EVALUATION OF MICRONIZED LENTIL AND ITS UTILIZATION IN LOW-FAT BEEF BURGERS

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ABSTRACT

Dehulled seed from four lentil market classes (large- and small-sized green and red types) were tempered to15% moisture and micronized to a surface temperature of 135 °C, and their compositional, physical, and functional properties were investigated. Micronization of lentil modified starch- and protein-related properties. Approximately 2.5 to 5.6% of the starch was gelatinized following micronization. Differential scanning calorimetry (DSC) results showed a 13 to 40% decrease in heat enthalpy, and viscosity analysis (Rapid Visco Analyzer) showed a 21 to 55% increase in peak viscosity and a 1 to 3 °C reduction in pasting temperature. Nitrogen solubility decreased across the pH range of 2 to 9, and lipoxygenase activity was reduced by 100-fold. There was a 25 to 43% increase in water holding capacity with no change in oil absorption capacity. The colour intensity of the pigments in the green and red lentil were reduced upon micronization of seed, and the particle size of flour was lowered with 7 to 13% more flour passing into the finest (<75 um) sieve.

Flours from dehulled green and red lentil (large type) were incorporated as a binder into low-fat (<10%) beef burgers at levels of 6 and 12%. Cooking properties, colour, texture, oxidative status, and sensory properties of these burgers were analyzed. Overall, increasing binder addition to low-fat beef burgers increased cooking yield up to 86% and minimized dimensional shrinkage upon cooking. Storage of raw, fresh burgers for 7 days under simulated retail display (4 °C) resulted in gradual reductions in HunterLab a* values, with those containing micronized lentil flour generally displaying significantly greater retention of redness from days 1 to 5 of storage. Thiobarbituric acid reacting substances (TBARS) of burgers containing micronized lentil flour were significantly lower compared with those containing non-micronized lentil after 9 to 11 weeks of frozen storage. A trained sensory panel (n=13) reported increasing burger juiciness and tenderness with the incorporation of up to 6% and 12% of lentil flour, respectively. Although off-flavour increased in burgers with non-micronized lentil flour addition, it was significantly reduced when seed was micronized. Consumer panel analysis (n=107) showed higher acceptability for burgers containing 6% micronized lentil flour or toasted wheat crumb compared with those containing non-micronized lentil flour or no binder. These results demonstrate that the conditions used to micronize

lentil altered the functional properties of the flours, and when used as a meat binder at an optimal level improved cooking properties, texture, and flavour profiles in low-fat beef burgers.

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1. INTRODUCTION

The demand for healthier alternative diets and ingredients is increasing. Saskatchewan is a large producer of lentil, 1,043 kilotonnes in 2008, of which over 90% was exported (Statistics Canada, 2009). As a result of its high protein (24.3 – 30.2%) and low-fat (1.0 – 1.3%) contents (Wang & Daun, 2006), there is an increasing trend to promote domestic use of lentil through value-added processing. While consumer demand for low-fat and high-protein foods is eminent, the use of lentil in product development could provide new opportunities for the Canadian pulse industry. Characteristics of lentil components and their functional behavior must be measured to assess its potential as a food ingredient. Moreover, any functional advantages must match the technical requirements of different product applications in order for lentil to be valuable as a food ingredient.

Micronization is a continuous dry heating process in which infrared radiation is applied to a product on a vibrating bed (Zheng et al., 1998) and has been employed in the food/feed industry to increase food safety, shelf stability, and nutritional value, and to decrease cooking times of starch-rich grains/seeds (Arntfield et al., 2001; Melcion & Valdebouze, 1977; Metussin et al., 1992; Zarkadas & Wiseman, 2002). With these apparent benefits, micronizing lentil seed can enhance their quality, while also influencing some functional properties, thus optimizing their potential applications in food.

Burgers and other comminuted meat products have become popular food items worldwide and efforts have been concentrated on reducing their fat content while also minimizing meat costs. The use of binders to replace meat in burger formulations is commonly practiced and can improve the cooking and sensory characteristics of the product. For example, toasted wheat crumb is commonly used as a binder in burger

production in Canada (CFIA, 2009). However, with wheat being a recognized allergen (CFIA, 2009), there is need to assess new alternative binders that can offer similar nutritional and functional benefits without causing allergic effects. Because lentil is high in protein and starch, and low in fat, they can serve as a binder in burgers.

The objectives explored in this study were:

1) To investigate the effects of micronization on the compositional, physical, and functional properties of lentil flour and to compare these properties to soy and wheat flours.

2) To incorporate lentil flours into low-fat beef burger formulations and to monitor the cooking, colour, texture, oxidative status, and sensory properties of these products.

3) To assess consumer acceptability of low-fat beef burger formulations containing lentil flour.

2. LITERATURE REVIEW

2.1 Lentil seed

2.1.1 Canadian lentil

The origins of lentil date back to the ancient times in Turkey and other Middle Eastern countries, and did not play a significant role in Western Canada until the 1970s (Saskatchewan Pulse Growers, 2010). Efforts in genetic research by the Crop Development Centre at the University of Saskatchewan have produced a more diverse and well-adapted cultivar base catering to specific market demands for lentil seeds and contributing to the commercial acceptance of Canadian lentil varieties (Saskatchewan Pulse Growers, 2010). Lentil (*Lens culinaris Medik.*) is a dicotyledonous legume plant bearing edible seeds. As of October 1, 2009, there were 40 registered lentil varieties in Canada (CFIA, 2009). This has led to the categorization of lentil seed by market classes based on cotyledon colour and size. Some designations (with corresponding cultivar names) include small green (*CDC Lemay, CDC Viceroy*), extra small red (*CDC Imperial, CDC Rosetown*), small red (*CDC Redberry, CDC Impact, CDC Maxim*), medium red (*CDC Red Rider, CDC Imax*), and large red (*CDC Robin*) (Crop Development Centre, Saskatoon, SK).

There is international demand for Canadian lentil. Canada produced 1,043 kilotonnes of lentils in 2008 (Statistics Canada, 2009), of which over 90% was exported to countries which included Spain, Turkey, Colombia, Egypt, Algeria, Sri Lanka, and India, comprising 44% of the world lentil exports. This significant contribution to the world lentil market can be attributed to efforts invested in plant genetics, agronomic strategies, and the use of mechanization in field activities (FarmFacts, 1998). Although, there is incentive to export Canadian lentil, various organizations promote domestic use

of lentil due to their high nutritional value. Canada's Food Guide (Health Canada, 2007) recommends consumption of 175 mL of cooked legumes to provide one serving from the *milk and alternatives* category per day. In addition, the Saskatchewan Pulse Growers Association (Saskatoon, SK) and Pulse Canada (Winnipeg, MB) promote initiatives for novel uses of legume crops involving processing.

2.1.2 Nutritional quality

Lentil can play a major role in human nutrition. The proximate composition of a typical Canadian lentil variety (non-dehulled) is 24.3 to 30.2% protein, 54.2 to 62.5% carbohydrate, 1.0 to 1.3% fat, and 2.3 to 3.5% ash, on a dry weight basis (Wang & Daun, 2006). Lentil is considered to be a good source of protein. However the presence of certain anti-nutritional factors can decrease protein digestibility, thus lowering the bioavailability of proteins from this plant source. Some examples of anti-nutrients present in lentil are trypsin inhibitors, tannins, oligosaccharides, and phytate (Costa et al., 2006; Sanz et al., 2001).

Trypsin inhibitors bind and inhibit the enzyme trypsin, consequently inhibiting protein digestibility and reducing the availability of amino acids (Kakade et al., 1974). Similarly, tannins bind with lysine or methionine thereby decreasing the bioavailability of these essential amino acids during digestion (Davis, 1981). Phytic acid binds to create a phytate-mineral-protein complex and can decrease the bioavailability of essential minerals including zinc, magnesium, phosphorus, calcium, and iron (Deshpande & Cheryan, 1984). Moreover, oligosaccharides present in legumes, predominantly in navy bean, red kidney bean, field pea and, to a lesser extent in lentil, are responsible for flatulence (Fleming, 1981). Work has been conducted to determine the distribution of these anti-nutrients within legume seeds. Although there is variation among different legumes, trypsin inhibitor activity and phytic acid are more prevalent in the seed cotyledon, whereas tannins are more concentrated within the seed coat in dry bean (Deshpande et al., 1982) and lentil seed (Wang, 2008). These distributions are important to consider when seeds are processed or fractionated for further use.

In order to optimize the nutritional potential of legume seeds, it may be necessary to remove antinutritional factors. Some processes that have shown to reduce anti-nutritional factors in the seed include soaking, boiling, dry heat roasting, micronization, pressure cooking (autoclaving), germination, fermentation, ethanol extraction, and dehulling (Frias et al., 1995; Ghavidel & Prakash, 2007; Khattab and Arntfield, 2009; Sanz et al., 2001; Urbano et al., 1995; Vidal-Valverde et al., 1994).

2.1.3 Lipoxygenase activity

Lipoxygenase (LOX) enzymes are ubiquitous in plant organelles at various concentrations with activities depending on pH conditions (Loiseau et al., 2001). Among various crops, legume seeds are known to inherently possess a high level of lipoxygenase activity, with soybean containing the greatest amount, comprising 1 to 2% of the total protein content (Loiseau et al., 2001). Lentil and cowpea crude protein extracts were also shown to contain high levels, up to 24,000,000 units/mg and 12,610,000 units/mg, respectively (Chang & McCurdy, 1985).

Lipoxygenase or lipoxygenase-like enzymes are thought to be responsible for lipid-derived off-flavours in legumes. Specific volatile aldehydes and alcohols (*e.g.* 2-*n*-pentylfuran and 3-*cis*-hexenal) oxidized from linoleic and linolenic acids have been identified as contributors to the green-beany flavour of soybean (Iassonova et al., 2009; Sessa, 1979). This oxidation can occur during seed storage, alkaline extraction, or storage of protein isolate, and can have quality implications. Addition of heat, acid, alcohol, or antioxidants can inhibit or inactivate lipoxygenase activity (Iassonova et al., 2009; Sessa, 1979).

Conditions for thermal inactivation of lipoxygenase are variable, depending on the heat source. Gordon and Mtebe (1987) studied the effect of temperature on lipoxygenase in winged bean and found that half of its activity was lost at 65 °C in 1 min, or eliminated when seed was boiled in water for 10 min. In addition, Al-Obaidy and Siddiqi (1981) observed a 40% loss of lipoxygenase activity in a broad bean crude extract between 55 and 65 °C and complete inactivation when exposed for 10 sec at

75 °C. Also, Busto et al. (1999) observed the half-life of pea lipoxygenase to be approximately 2 min at 70 °C. Kouzeh-Kanani et al. (1982) demonstrated 95.5% inactivation of LOX in soybean within 60 sec of infrared treatment.

Significant variations in reported lipoxygenase values exist in legumes and are commonly attributed to differences in quantitative techniques involving sample preparation, enzyme extraction conditions, substrate conditions, use of surfactant, and assay conditions (Chang & McCurdy,1985). The distribution of lipoxygenase activity in pea was found to be greater in the cotyledon (92%) and lower in the hull (5 – 8%), (Eriksson, 1967), and Al-Obaidy and Siddiqi (1981) found dehulled broad bean to contain 50% less lipoxygenase activity than the intact seed.

Various methods exist for quantifying lipoxygenase activity, including those based on oxygen uptake, formation of conjugated dienes (spectrophotometric), coupled oxidation of β-carotene, and determination of hydroperoxides (Grossman & Zakut, 1979). Oxygen uptake methods are advantageous over those measuring conjugated dienes in that turbid samples can be analyzed. In addition, solubilizing the unsaturated fatty acid substrate at alkaline pH proved a challenge for the spectrophotometric methods. However, the use of detergent such as Tween 20 solved this problem (Surrey, 1964). Another disadvantage of the spectrophotometric assays is that the presence of polyphenolic compounds can interfere with the absorbance reading (Loisseau et al., 2001). The main drawbacks of the coupled oxidation and hydroperoxide determination methods include the lack of or limited linear relationships between enzyme and substrate, as well as carotene solubility in the former assay. Despite these abovementioned shortcomings, the spectrophotometric assay is the most commonly used to quantify lipoxygenase activity in plant foods due to its high precision (Al-Obaidy & Siddiqi, 1981; Busto et al., 1999; Chang & McCurdy, 1985; Kumar et al., 2006).

2.2 Lentil processing

2.2.1 Cleaning and dehulling

Lentil seed is comprised of the seed coat (8%), cotyledon (85%), and embryo (2%) (Sokhansanj & Patil, 1995). Dehulling is commonly performed for specific market classes. Dehulling efficiency can be optimized through various techniques such as tempering and using a uniform seed size that is most compatible with the dehulling equipment. Erskine et al. (1991) concluded that a 4 mm seed size, a 1 minute immersion time, and a tempering time of 24 hours to attain 12.8% final moisture were optimal conditions for dehulling lentil. The dehulling was performed on a laboratory scale dehuller comprised of 2 horizontal round stones. Similarly, Wang (2008) employed a tempering for 24 hours to attain 12.5% moisture in lentil seeds of 4.5 to 5.0 mm diameter to be used in the study of varietal influences on dehulling efficiency.

Dehulling legume seed influence their chemical composition. Increases were found in protein and thiamin contents, *in vitro* iron and calcium availabilities, and *in vitro* starch and protein digestibilities, wherease decreases in phytic acid and tannin contents were observed in legumes (Ghavidel & Prakash, 2007). Dehulling legumes prior to further processing or for direct market sale can optimize nutrition, functionality, and aesthetics.

2.2.2 Milling flour

Milling of legume seed is conducted to produce legume flours for commercial sale, or to prepare samples for further fractionation. Common dry mills for grinding legume seeds include the plate mill employing two parallel plates moving in opposite directions (Singh et al., 2005), the pin mill which involves the passing of coarsely ground flour through rotor pins, and the Wiley mill which involves a set of blades moving in a centrifugal motion. The resulting grind size and particle size distribution can vary depending on the type of mill used as well as the conditions of milling, and any sample pre-treatments (Singh et al., 2005). Wolcott (1977) compared the Sonic Sifter and Ro-tap equipment used for analyzing the particle size distribution of sediment and

found the former to have less sample loss, and to be more quiet, portable, and versatile despite its lower precision.

2.2.3 Heat-treatment by micronization

Micronization is a continuous heating process in which infrared radiation (λ = 1.8 – 3.4 μm) is applied to a product on a vibrating bed (Zheng et al., 1998). This dry, heat process involves the absorption of electromagnetic waves by the material effectively generating a high temperature for a short duration. In general, complex food components (water, protein, starch, and lipid) will absorb radiative energy most efficiently in the far infrared (3 – 1000 μm) region (Sandhu, 1986), leading to molecular vibration and radiative surface heating.

Micronization of plant materials (*i.e.* seeds) has been employed in the food/feed industry to achieve various objectives. These include increasing food safety and shelf stability through the reduction of microbial activity and enzyme inactivation, altering of physiochemical properties to achieve functional ingredients, and optimizing nutritional value through inactivation of antinutritional factors (Melcion & Valdebouze, 1977; Metussin et al., 1992). Some current applications include starch-rich foods with reduced cooking times (Arntfield et al., 2001), dehydration of fruits, vegetables, seafood, and pasta through continuous tunnels, and as a pre-treatment blanching for the frozen, packaged vegetable market (Krishnamurthy et al., 2008). Because the infrared radiation from micronization will interact with water, protein, starch, and lipid molecules to create heat, there will be influences on nutritional, structural, physiochemical, and sensory quality of food materials (Krishnamurthy et al., 2008).Effect of micronization on nutrition

Micronizing conditions can influence the activity of anti-nutrients. A 31% decrease in trypsin inhibitor activity in lentils micronized to 140 °C was observed by Fasina et al. (2001). The effect of various heat treatments, including infrared heating on antinutrients in cowpea, pea, and kidney bean were studied by Khattab and Arntfield (2009) where they generally found that micronization (24% moisture, 90 °C)

significantly reduced the levels of antinutritional factors such as tannins, phytic acid, trypin inhibitor, oligosaccharides. However, Khattab and Arntfield (2009) found also that the ability of micronization to reduce antinutritional factors was less than that of boiling, roasting, autoclaving, or microwaving.

Positive effects of micronization on enzyme inactivation have been demonstrated. Because the lipoxygenase enzyme is present in various legumes and can oxidize polyunsaturated fats and cause off-flavour development, inactivation of this enzyme is critical prior to any long-term preservation. Far infrared energy technology has already been employed on pea and carrot as a pre-treatment prior to freezing (Galindo et al., 2005; Van Zuilichem et al., 1986). Due to its associated high energy efficiency, with minimal cell damage to the vegetable, use of infrared radiation can serve as a viable substitute for blanching in the frozen vegetable industry (Krishnamurthy et al., 2008).

In addition to food spoilage issues, micronization demonstrates potential as a tool for food safety. Microorganisms such as pathogenic bacteria, spores, yeast, and mould can be inactivated by micronization (Krishnamurthy et al., 2008). However, due to the specific inactivation requirements of different organisms, examination of how variation in micronizing parameters affect each pathogen is necessary. These parameters include micronizing power, food temperature, peak wavelength, sample depth, and moisture content (Krishnamurthy et al., 2008). Mechanisms for inactivation include damage to the DNA, RNA, ribosome, cell envelope, and proteins in the microbial cell (Hamanaka et al., 2006), and the inactivation is influenced by association with water molecules.

2.2.3.1 Effect of micronization on structural properties

Seed tempering to produce specific moisture levels significantly influences the effects of micronization on textural properties. As a result of tempering prior to micronization, lentil has a weaker structure due to greater porosity (Arntfield et al., 1997; Fasina et al., 2001). From these studies, tempering involving moisture levels ranging from 25 to 33% prior to micronization to a surface temperature of 140 ºC led to

increased starch gelatinization. Higher seed moisture levels are required for starch granules to swell, while proteins become more susceptible to denaturation and aggregation during micronization (Arntfield et al., 1997). Ultimately, tempering to the optimal moisture level prior to micronization can reduce hardness and cooking times, which can enhance the marketability and utilization of various crops.

2.2.3.2 Effect of micronization on physiochemical properties

The physiochemical qualities of seed flours are important attributes when considering their potential as functional food ingredients. Examples of some functional properties include nitrogen solubility, water holding capacity, oil absorption capacity, swelling index, gelation, and foam capacity. Application of heat to seeds has been found to increase water absorbing capacity, least gelation concentration, and surface hydrophobicity, and decrease water solubility index, swelling index, gel strength, and foam capacity in the case of micronizing cowpea flour to 130 °C (Mwangwela et al., 2007). Mwangwela et al. (2007) concluded that a high micronization temperature (170 °C) could be detrimental to overall protein quality with respect to functional food applications, and that there is potential for using milder conditions to process the flour to achieve more ideal physiochemical properties.

Micronization generally decreases nitrogen solubility of legumes over a range of pH. Zheng et al. (1998) studied the effect of micronization at 140 ºC on nitrogen solubility of several legumes, including lentil, and concluded that higher micronization temperatures further reduced nitrogen solubility of the flour over pH 2 - 12. They suggested that denaturation and hydrophobic aggregation had occurred as a result of micronization. Flour or protein isolate from lentil was found to have the lowest nitrogen solubility and greatest degree of denaturation at pH 4 - 5, regardless of whether the samples had been micronized or not (Bora, 2002; Ghavidel & Prakash, 2006; Zheng et al., 1998).

Other techniques used for assessing the thermal impacts on starch or protein components involve viscometry and calorimetry. Starch granules can become

gelatinized in the presence of excess moisture and heat. Rapid Visco Analyzer (RVA) and differential scanning calorimetry (DSC) analysis are methods used to detect changes in seed components as a function of temperature. The degree of gelatinization can also be measured quantitatively using enzyme reactions involving amyloglucosidase (Chiang & Johnson, 1977). Using this method, Arntfield et al. (1997) detected 29% gelatinized starch when lentil was pre-tempered to 25% moisture, which increased to 68% gelatinized starch when tempered to 33% moisture.

The RVA can be used to monitor viscosity changes in a slurry as it is subjected to a heating program. Parameters obtained from viscograms include peak viscosity, breakdown, peak time, and pasting temperature. Peak viscosity for lentil (11.9% flour slurry) was determined to be between 1185 to 1359 cP (Chung et al., 2008) and indicated the water-binding capacity of the slurry. Chung et al. (2008) found their prepared lentil slurry to initiate pasting at 70 °C.

DSC measures the energy required to establish a zero temperature difference between a substance and a reference material against either time or temperature, as the two sample cells are subjected to heating programs of identical rate and intensity (Nielsen et al., 1998). In the case of foods, DSC has been employed for detecting starch gelatinization and protein denaturation, and oil melting points. Parameters include onset temperature (T_o, °C), peak temperature (T_d, °C), heat of enthalpy (ΔH, J/g). Gelatinization of lentil flour or starch extracts has been found to occur at 64 to 68 °C (Biliaderis et al., 1980; Chung et al., 2008), whereas the peak temperature related to protein denaturation ranges from 92 to 99 °C depending on the purity of the protein extract (Lee et al., 2007; Sosulski et al., 1985).

2.2.3.3 Effect of micronization on sensory characteristics

The effect of micronization on sensory properties is often considered. Colour is a criterion for assessing the market value and acceptability of lentil. The effects of micronizing conditions on lentil colour have been documented. Micronizing green lentil up to 170 °C resulted in lentil seed that was darker, less green, more red, and less yellow (Arntfield et al., 2001). In comparison with other legumes, micronized cowpea (white), and navy and black beans also darkened, and had lower redness and yellowness with increasing micronization temperature (Bellido et al., 2006; Mwangwela et al., 2007). In general, this darkening effect on seeds was attributed to Maillard browning involving the reaction between reducing sugars and protein. In an earlier publication by Arntfield et al. (1997), the higher moisture conditions in the seed resulted in the development of even darker pigments.

Development of aroma is also a quality consideration in micronized seeds. The freshness of flour from seeds with a high fat content, such as soy, was maintained for one year as a result of micronization (Kouzeh-Kanani et al., 1982). Unmicronized samples in this study resulted in rancidity development. In the case of air-classified pea protein concentrate, bitterness was reduced, or flavour was more bland when made from micronized pea (107 or 117 °C) compared with protein concentrate produced from raw pea (McCurdy, 1992). Prevention or removal of off-flavours through micronization is an advantage for ingredients to be used in food applications.

2.3 Meat Burgers

Comminuted beef products are a popular form of processed meat in Canada. In 2001, beef was the meat that had the highest annual food expenditure ($106 per capita) in Canada, followed by chicken ($85 per capita), and pork ($41 per capita) (Statistics Canada, 2002). Of the total value of beef purchased in Canadian retail stores, 35% was in the form of ground beef or patties (Statistics Canada, 2002). A meat burger is a comminuted, pre-formed meat product with an individual (raw) serving size of approximately 80 - 130 grams (CFIA, 2009). Meat burgers can be prepared in the home

or produced for retail, and conveniently sold in fresh, frozen, or pre-cooked forms. Common sources of meat used for burgers found in the Canadian market include beef, turkey, chicken, pork, and lamb. Other meat sources such as ostrich (Fernandez et al., 2006) and buffalo (Modi et al., 2003) have also been investigated in research studies.

2.3.1 Low-fat burgers

Commercially available burgers are typically manufactured to contain approximately 15 to 30% fat (UDSA, 2009). Fat plays important functional and sensory roles in meat products. For example, ground beef patties containing 28% fat received the greatest juiciness and tenderness scores, while scores progressively decreased as fat levels decreased to 16% (Cross et al., 1980). However, there are nutritional concerns with high fat levels in burgers, as well as other considerations such as increased splattering or drip loss upon cooking, or greasy mouth feel (Cross et al., 1980; Sheard et al., 1998). For these reasons, the demand for low-fat foods is rising. In catering to this popular low-fat trend, functional and sensory properties affected by the removal of fat in meat products will need to be considered. Low-fat beef burgers produced for scientific studies range between 3.7 and 10% fat (Table 2.1). Despite the advantage of the lower fat loss found in the cooking of a lower fat burger, it has been established that simple removal of fat from meat formulations produces a burger with lower juiciness, tenderness, and flavour scores, and can lead to lower acceptability scores (Berry, 1993). Therefore, an agent that can help to retain or enhance sensory quality can be investigated to achieve a more healthy and palatable low-fat meat burger.

Table 2.1: Examples of various binder additions to comminuted meat products found in the literature.

Author	Binder Type	Treatment	Use level	Meat System
Serdaroglu et al., 2005	Lentils, chickpea, blackeye bean flour	Soaked 12 h, boiled 1.5 hr, dried, ground	10%	Meat balls (9% fat)
Prinyawiwatkul et al., 1997	Cowpea, peanut flour	Fermented, defatted	0-20%	Chicken nuggets
Modi et al., 2003	Bengal, green, black gram flour	Dehulled, roasted 5 min at 150 ºC	8%	Buffalo burger
Dzudie et al., 2002	Common bean flour		2.5-10%	Beef sausage
Shaner & Baldwin, 1979	Chickpea meal flour	Defatted, 30% hydrated		Meat loaf
Ulu, 2004	Wheat flour, whey concentrate, soy protein isolate	Concentrate, isolate	0.20%	Meat balls
El-Magoli et al., 1996	Whey protein concentrate		1-4%	Low-fat beef burgers (10% fat)
Hale et al., 2002	Whey protein	Extrusion with cornstarch, then rehydrated (1.5:1)	0-50%	low-fat beef burgers (10% fat)
Kumar & Sharma, 2004	Carageenan		0.25-0.75%	Low-fat pork patty (<10% fat)
Turhan et al., 2005	Hazelnut pellicle		1-5%	Low-fat beef burgers (10% fat)
Besbes et al., 2008	Pea fibre, wheat		0.5-1.5%	Beef burger (12.5% fat)
Yilmaz & Daglioglu, 2003	Oat bran		5-20%	Veal meat ball (8-21% fat)
Lui, 1999 (M.Sc.Thesis)	Barley meal	Micronized 125 °C	5%	Low-fat pork burgers (5% fat)

The use of fillers or extenders in burger formulations to partially replace meat is a common practice and has been shown to improve texture and flavour, in addition to having nutritional benefits. Examples of quality characteristics in meat burgers upon cooking include fat and moisture loss, leading to shrinkage and, consequently, toughness. Incorporation of certain levels of low-fat binders into low-fat meat products has resulted in various improvements relating to cooking yield, burger dimensions, and texture properties. Desmon and Troy (1998) compared 17 commercially available non-meat adjuncts (carrageenan, locust bean gum, pectin, maltodextrin, tapioca starch, soy protein, whey protein, blood protein, oat fibre, egg albumin, sodium alginate, calcium lactate) from the USA, UK, Ireland, Netherlands, Denmark, France, and Germany at 0.5 to 5.0% use-levels in the manufacture of low-fat beef burgers. In this study, beef burgers containing pectin, cellulose, oat fibre, and carrageenan produced the highest flavour and overall quality scores. Other examples of fillers, extenders, or binders used in various meat products are shown in Table 2.1.

In contrast to the traditional meat patty, burgers are permitted under Canadian regulations to be formulated with fillers (Meat Inspection Regulations, 1990). The Canadian Food Inspection Agency requires that meat burgers contain a minimum of 13 and 15% total protein in the raw and cooked state, respectively. In addition, a minimum of 11.5% and 13.5% of the protein in the raw and cooked state, respectively, must come from a meat source (Meat Inspection Regulations, 1990). Therefore, these limits must be considered when adding non-meat constituents as extenders to meat burgers. Given these protein requirements, incorporation of up to 20% binder (hydrated or non-hydrated) has been researched in various meat products using flours from high-protein legumes (Hale et al., 2002; Prinyawiwatkul et al., 1997; Yilmaz & Daglioglu, 2003). Toasted wheat crumb is a meat binder/extender commercially used in Canada. It is made by cooking dough (wheat flour and water) that has been enzymatically (yeast) or chemically leavened (CFIA, 2009). The hardened dough is then crumbled to a desired particle size. Despite its effectiveness and recognition as a meat binder, wheat is considered a high priority allergen in Canada (CFIA, 2009). Therefore, lentil flour can have potential as a binder in a meat burger system.

2.3.2 Lentil applications in food

Because lentil is high in protein and carbohydrate, and low in fat, it can be used as a functional ingredient in a range of food applications. Various studies have investigated the efficacy of incorporating lentil and lentil fractions into food systems. Different fractions of the lentil bean that have been utilized include the hull, dehulled fraction, and extracts such as protein. Reasons to explore such applications are to improve product functionality and nutritional value. Also, it may increase economy by lowering costs through displacement of more expensive or scarce ingredients, and adding value to both the lentil and the specific food processing industry. Examples of lentil applications in various food systems include using raw lentil flour for spaghetti noodle formulation (Zhao et al., 2005) and expanded snack wafers (Hardacre et al., 2006), lentil hulls (3, 5, and 7%) used to fortify bread (Dalgetty & Baik, 2006), and lentil protein concentrate/glycerin used to synthesize an edible film (Bamdad et al., 2006).

Due to the protein and specific textural requirements of meat products, incorporating plant-based binders into meat systems has been explored. Examples of binders studied originate from different components of legumes, cereals, grains, nuts, or seaweed (Table 2.1). Since a protein concentrate is more pure than a flour, it enables one to study more easily the effects of specific flour components. However, the use of a minimally processed flour containing starch and protein serves as a natural first step for investigating the potential of novel ingredients. This is relevant in the case of seeds from legume crops where their application as functional food ingredients is a fairly current development. Further investigation of the individual contributions of protein or starch components could follow. Examples of meat applications include meat balls, loaf, burgers, sausages, and chicken nuggets (Table 2.1). Notable advantages include increased water holding capacities leading to superior cooking properties, and improved textural and nutritional qualities. However, flavour and colour attributes tend to be negatively affected when flours are incorporated at higher use-levels, as in the case of a 20% cowpea and peanut flour blend in chicken nuggets (Prinyawiwatkul et al., 1997).

2.3.2.1 Effect of plant binders on meat oxidative stability and quality

The addition of foreign ingredients to meat products can affect meat oxidative stability leading to colour and flavour changes. These quality changes can be attributed to protein and lipid oxidation.

2.3.2.1.1 Myoglobin oxidation

The red pigment characteristic of raw meat is due to the hemoglobin contained in blood or myoglobin present in muscles. Because most of the blood is drained during animal slaughter, 80 to 90% of the red meat colour will be contributed by myoglobin (Aberle et al., 2001). Myoglobin is a globular protein containing a central heme ring in which the oxidative status of the iron determines the meat colour. For example, when iron is oxidized (ferric, Fe^{3+}), it cannot bind oxygen, and the myoglobin will be converted into metmyoglobin exhibiting a brown colour. Conversely, when iron is reduced (ferrous, Fe^{2+}), the pigment will be converted to deoxymyoglobin in the absence of oxygen, exhibiting a purplish red colour, or converted to oxymyoglobin in the presence of oxygen, exhibiting a bright red colour (Aberle et al., 2001).

Myoglobin oxidation occurs as excess oxygen is available (Aberle et al., 2001). As meat is ground or processed into smaller pieces, the surface area is increased and the rate of oxidative reactions thus increases. Moreover, addition of foreign ingredients such as binders or salt can influence these oxidative rates. In raw meat, products of lipid oxidation can initiate the oxidation of the heme iron of myoglobin into its ferric state (Yin et al., 1993). Similarly, in cooked meat, conversion of iron into its ferric state occurs as the myoglobin protein becomes heat denatured, releasing the pro-oxidant iron into the muscle. As iron converts to its ferric state, oxymyoglobin is converted into metmyoglobin and is accompanied by a red to brown colour change. Ganhao et al. (2010) reported increases in oxidative degradation of muscle proteins in cooked and refrigerated pork burgers (<2.2% fat) as increases in protein carbonyl content were measured over time. Protein oxidation in the study of Ganhao et al. (2010) was accompanied by reductions in HunterLab redness (a*) during 12 days of refrigerated

storage of the cooked product. These authors state that the impact of protein oxidation was likely more significant than that of lipid oxidation due to the protein content being 10-fold higher than the lipid content in the pork burgers.

2.3.2.1.2 Lipid oxidation

Lipid oxidation occurs in meat when it is exposed to oxygen and produces aldehydes, acids, and ketones, which contribute to rancid off-flavours. Polyunsaturated fats or phospholipids are more susceptible to auto-oxidation, with increasing rates under pro-oxidant conditions (metal ions, heat, UV, low pH) (Aberle et al., 2001). Auto-oxidation can be separated into three stages. The first is an initiation stage where a hydrogen is abstracted from a fatty acid creating an alkyl radical. The second is a propagation stage where oxygen will react with the alkyl radical yielding a very high energy peroxyl radical, which can abstract another hydrogen from another fatty acid thus propagating a chain reaction. Hydroperoxide compounds can further break down into low molecular weight compounds that exert a rancid odour. Under low oxygen conditions, termination of auto-oxidation can occur where the two alkyls react, or an alkyl and peroxyl radical react (Damodaran et al., 2008). Primary lipid oxidation products are those resulting from initiation and propagation, whereas secondary lipid oxidation products are those resulting from further breakdown of these byproducts into more volatile compounds.

Because lipid oxidation by-products are produced in stages, their production can be monitored by measuring levels of substrate or oxygen, oxidants, and primary or secondary products (Shahidi & Zhong, 2007). Methods used to analyze oxidative status of raw/cooked comminuted meat products over time (or point in time) include measuring dynamic head space (El-Magoli et al., 1996), hydrogen peroxide using peroxide value (initial stage) (Chen et al., 2008), conjugated dienes or trienes using spectophotometric techniques (primary by-products), malonaldehyde using thiobarbituric acid reacting substances (TBARS) test (secondary) (Carbonell et al., 2005; Chen et al., 2008; Fernández-Lopez et al., 2006; Georgantelis et al., 2007; Kumar & Sharma, 2004; Modi et al., 2003), and *p*-anisidine value (secondary by-products)

(Rodriguez-Estrada et al., 1997). Antioxidants can be applied to prevent or delay auto-oxidation through radical scavenging or metal chelation mechanisms (Shahidi & Zhong, 2007).

Studies have looked at the effect of plant-derived meat extenders in meat applications on microbial quality and oxidative stability. For example, Rhee et al. (1985) studied ground beef processed with and without cottonseed flour and measured aerobic count, discolouration, and lipid oxidation over six days. They found that ground beef containing cottonseed flour had a higher count initially but was the same as untreated ground beef after 3 - 6 days. This decrease in microbial count could be attributed to the antioxidant effect of phenolics or oxygen scavenging action in cottonseed flours. Discolouration and lipid oxidation (TBAR) was reduced in ground beef containing cottonseed flour. Similarly, beef burgers extended with soy flour or concentrate had a lower incidence of rancid flavour according to a trained sensory panel (n=10) compared with an all beef control or when soy isolate was used.

2.3.2.2 Texture of foods

2.3.2.2.1 Instrumental analysis

Textural quality is often considered in new food product development. The use of mechanical instruments to objectively measure food texture is a more economical alternative to conducting sensory studies, and is often used in conjunction with sensory data to strengthen the results (Lawless & Heymann, 1998). Shear force and texture profile analysis are two mechanical methods used for studying the texture of various foods. Although instrumental techniques are convenient, there is large variation in the data obtained from shear force or texture profile analysis (TPA) methods due to different experimental factors apparent in sample preparation or test conditions (Szczesniak, 1962), making it difficult to compare absolute data in foods produced from different laboratories. Because different foods have unique physical characteristics, technical factors for instrumental setup need to be modified to suit each food type.

Shear force analysis is an empirical texture test that measures the force (N) to puncture or shear a food (Bourne et al., 1978). A force-time curve is produced where peak force can be obtained from the resulting curve representing the initial hardness of a sample. Developments have been made in instrumental tests which imitate conditions that a food is subjected to in the mouth. Contrary to the single point shear force analysis, the Instron Universal Test Machine attempts to achieve this repeated force by compressing standard pieces of food two times (Bourne, 1968). The texture profile analysis produces a force-time curve with two distinct peaks where five texture parameters are measured and from which two parameters are calculated (Bourne et al., 1978). These parameters include fracturability, hardness, cohesiveness, adhesiveness, springiness, gumminess, and chewiness. Although this multi-point analysis yields several parameters that are effective in distinguishing characteristics within the same food type, it is necessary to adjust the texture press mechanical settings to cater to the specific food category (Bourne et al., 1978).

Correlations between instrumental texture and sensory results have been made for foods representing a spectrum of textures including nuts, soft cheese, fruits, vegetables, meat, hard candy, butter, and bread (Meullenet et al., 1998). Although the relationship between sensory and instrumental data for a given food type is seldom linear, correlations have been made (Szczesniak, 1987). Overall, it was found that the hardness and springiness of these foods were significantly correlated between sensory and instrumental TPA parameters, whereas cohesiveness and chewiness were not highly correlated between objective and subjective texture methods. Dransfield et al. (1984) studied the relationship between texture profile attributes and beef burger acceptability and reported that “ease of fragmentation” (cohesiveness), “degree of comminution” (meat particle size), “tenderness,” and “moistness” were the best predictors of overall acceptability in beef burgers. However, psychological factors, in addition to methodological factors, can also influence texture results (Meullenet et al., 1998).

2.3.3 Conducting sensory panels for meat products

The effective use of human subjects to assess food quality is becoming more recognized and is commonly practiced in food companies (Erhardt, 1978). It is necessary to conduct sensory studies to facilitate the development of new products or understand the effects of reformulation on sensory quality and consumer acceptability. Optimizing food processes that yield the most robust and desired food product will ultimately lead to market success. Sensory analysis can also be used for determining shelf life (Lawless & Heymann, 1998).

It is important to conduct sensory tests in a controlled environment with specific test objectives. This will dictate the sample and treatment types to be tested and suitable terms for the questionnaire. Procedures for conducting sensory tests include controlling all non-test variables (light, booths, odours, randomized sample codes, randomized serving and cooking order, standard sample temperatures, use of cracker and water or juice to cleanse palate) (Meilgaard et al., 2007).

2.3.3.1 Trained sensory panel (descriptive)

For a trained sensory panel, panelists are recruited, trained, and screened before introducing the panel to the final samples. Appearance, texture, and flavour attributes related to the specific food products are described using familiar terminologies. Panelists become acquainted with these terminologies through the use of training samples specially formulated to demonstrate each attribute at specific intensities. Trained panelists should be able to identify each attribute and assess the intensity using a common scale. The screening process will evaluate panelist performance based on accuracy and precision. Panelists are ranked based on their performance, leading to either elimination or further retraining of panelists (Lawless & Heymann, 1998; Meilgaard et al., 2007).

2.3.3.2 Untrained consumer panel (preference testing)

Conducting a consumer sensory analysis is helpful in understanding the characteristics of prospective consumers and the ultimately the potential market success for food products. These tests differ from those conducted by a trained sensory panel in its measure of the overall acceptability of products. Specifically, only differences in tenderness can be measured in either trained sensory panels or by instrumental techniques (AMSA, 1995).

Unlike the descriptions and intensities of sensory stimuli, there is inherent variation in consumer preferences or perceptions of a food product, particularly with respect to its taste and smell (Moskowitz, 1991). Therefore, the purpose of conducting sensory analysis using a consumer panel is to assess the acceptability of food samples according to a general audience. Because it is common for consumer acceptability tests to yield widely opposing outcomes among panelists, statistical analysis of means can cancel each other out (Beilken et al., 1991). To extract further valuable information from acceptance data, consumer segmentation has commonly been used. Categorizing consumers by specific demographic or behavioural characteristics can identify significant differences in acceptability within these subgroups. Consumer segmentation by gender, age, culinary habits, and aroma sensitivity was used in understanding the acceptability of boar meat (Furnols et al., 2003), and segmentation by preference of steak doneness in determining acceptability of steaks cooked to different end-point temperatures (Schmidt et al., 2010). History/experience and attitude of a consumer can influence hedonic acceptance scores (Deliza & MacFie, 1996) and therefore will also affect food choices. Understanding consumer segments that respond most positively to a food product can efficiently focus marketing efforts and resources (Meilgaard et al., 2007).

3. MATERIALS AND METHODS

3.1 Study I: Evaluating the effect of lentil type and heat treatment on functional properties of lentil flour

The functional properties of lentil flours as food ingredients may vary depending on their crop variety. Four market classes of lentil seed were micronized and ground, and their functional properties were analyzed and compared to some commercially available reference flours. This assessment will help to screen lentil types suitable for meat applications in the subsequent study.

3.1.1 Sample descriptions

3.1.1.1 Lentil seed

Four types of Saskatchewan-grown lentil (*Laird* - large green, *CDC Redberry* - large red splits, *French Green* - small green, *CDC Robin* - small red footballs) were harvested in 2007, cleaned, dehulled, and received from the Crop Development Centre, College of Agriculture and Bioresources, University of Saskatchewan in June and August, 2008. Cleaning and dehulling of lentil seed was commercially performed at Saskcan Pulse Trading (Regina, Saskatchewan). Incoming seed samples (50-kg sacks) were vacuum-packaged into 3-kg portions in polyethylene bags, and stored in enclosed containers at room temperature prior to use.

3.1.1.2 Reference flours

Three reference flours were used in this study. Pea flour (*Fiesta Flour*) was obtained from Parrheim Foods Ltd. (Saskatoon, SK). Wheat flour (Product Code 1020, containing hard wheat flour, fortified with niacin, iron, thiamine, riboflavin, folic acid),

and soy flour (Product Code 1028, defatted) were obtained from NewlyWeds Foods (Edmonton, AB). These flours were stored as described above.

3.1.2 Processing of lentil

3.1.2.1 Tempering and micronization of lentil

Prior to micronizing, approximately 4 - 5 kg of whole, dehulled lentil were tempered in polyethylene bags (40.6 cm x 30.5 cm) by adding a pre-determined amount of deionized water to the lentils (AACC 26-95, 1995):

$$W = [L(\text{Moisture}_T - \text{Moisture}_O)]/(100 - \text{Moisture}_O)$$

Where,
W = Water weight required (grams)
L = Lentil weight (grams)
Moisture_T = Moisture required at tempering (%)
Moisture_O = Moisture content of seeds before tempering (%)

The bags were heat-sealed and then shaken manually to evenly distribute the water. After sitting for 10 min, bags were re-shaken to disintegrate any clumps. The samples were allowed to temper at ambient temperature for 16 h to achieve an equilibrium final moisture content of 15%.

Dehulled lentil seed was heat-treated using micronizing technology available at InfraReady Products Ltd. (Saskatoon, SK). Lentil samples were micronized on a laboratory-scale micronizer (Model A 156379-B0, FMC Syntron® Bulk Handling Equipment, Homer City, PA) composed of a gas heating element with two sets of three ceramic tiles (Model type R 1603-2 PAT, Rinnai, Japan), a Syntron feeder (Model F010, Riley Automation Ltd., Derby, England) and a Syntron magnetic feeder (Model BF2 A, FMC Corporation, Homer City, PA). Tempered lentil seed was placed into a hopper which fed onto a moving vibrating bed exposed to overhead infrared lamps positioned 20 cm above. The conveyor belt speed and vibrating bed was adjusted so that a constant

seed temperature during final collection was achieved. A hand-held IR Temp Gun thermometer (Oakton, Vernon Hills, IL) was used to measure the surface temperature of outcoming seed. Lentil was micronized to a seed surface temperature of 130 to 135 °C by using micronizing rates of 143 g/min and 286 g/min for large and small type lentil, respectively. Micronized samples were thinly spread onto a table, allowing the seed to cool for 30 min at room temperature prior to packaging in ZipLock® freezer bags (S.C. Johnson and Son Limited, Brantford, ON) and subsequently in vacuum bags. Moisture content was determined using AOAC Method 925.10 (1990) before and after micronization.

3.1.2.2 Milling of lentil

Lentil samples were ground to pass a 0.5 mm screen using a Cyclone Sample Mill (UDY Corporation, Fort Collins, CO). All milled flour samples were vacuum packaged in polyethylene plastic bags and stored away from direct heat, light, and moisture.

3.1.3 Compositional and physical properties of flours

3.1.3.1 pH, moisture, fat, and protein analysis

The pHs of flours were measured using AOAC Method 943.02 (1990). To 10 g of flour, 100 mL of deionized water was added at 20 °C and blended for 1 min. The pH of the slurry was measured using a pH meter (Model 915, Fisher Scientific, Nepean, ON).

Moisture was analyzed using AOAC Method 925.10 (1990). Approximately 2.0 g of flour was weighed in pre-weighed aluminum pans. Prepared pans were placed in a drying oven at 100 - 102 °C. After 12 - 16 h, sample pans were placed in a desiccator and allowed to cool. Pans were re-weighed and loss was assumed to be moisture.

Crude fat was measured using AACC Method 30 -25 (1995). Fat was extracted from a 2 to 5 g flour sample using a Labconco Goldfisch fat extractor apparatus with petroleum ether for 5 h. Protein was analyzed using AACC Method 46-11 (1995).

Protein was calculated using a nitrogen to protein conversion factor of 5.70 for wheat and 6.25 for legumes. Ash content was measured using AOAC Method 923.03 (1990). All analyses were conducted in duplicate, with the exception of triplicate analysis of moisture.

3.1.3.2 Total starch

The total starch content of lentil was analyzed using a Megazyme kit (K-TSTA/05/06, Megazyme International, Wicklow, Ireland), according to AACC Method 76.13.

3.1.3.3 Gelatinized starch

Gelatinized starch content was analyzed using the method of Chiang and Johnson (1977), with some modifications. A flour sample (20 mg) was weighed into a 50-mL centrifuge tube and washed two times with 10 mL of 80% ethanol, vortexed, and centrifuged (1,500 x *g*) for 10 min at 21 °C. After decanting the ethanol, residual solvent was evaporated from the centrifuge pellets using a vacuum oven (30 °C at 3.33 kPa for 12 h). Dried starch pellets were dispersed in 5 mL of double deionized water and 25 mL enzyme solution was added, vortexed, and incubated at 40 °C in a shaking water bath (Lab Companion, Jeio Tech, Woburn, MA) at 100 rpm for 30 min. Then, 2 mL of 25% trichloroacetic acid was added to stop the reaction and the samples were centrifuged at 16,000 x *g* for 5 min. A glucose standard curve (0.25, 0.50, 0.75, 1.0 and mg/mL) was prepared at this time. Then, 0.1 mL of supernatant or glucose standard was transferred to test tubes, mixed with 3 mL of *o*-toluidine reagent (1.5 g thiourea was dissolved in 940 mL glacial acetic acid and then 60 mL o-toluidine was added), and incubated in a water bath at 100 °C for 10 min.

Absorbance was measured at 630 nm on a Spectronic Genesys Spectro-photometer (Milton Roy Co., New Rochelle, NY) and gelatinized starch (%), G, was calculated using the equation, G = ((D-K)/TS)(100), where D (%) = starch digested by amyloglucosidase, K = correction factor, and TS = total starch (%). K was calculated by weighing out and digesting 5, 10, 15, and 20 mg of native lentil flour, and determining

the glucose released. A curve was developed where the glucose released was linearly related to a corresponding amount of total starch. K = slope x 100.

3.1.3.4 Particle size distribution of lentil flour

The particle size distribution (PSD) of lentil flour was analyzed using an Allen-Bradley Sonic Sifter (Allen-Bradley, Milwaukee, WI), which involves a combination of vertical oscillation of air and mechanical pulses applied to the stack of sieves containing the flour to be sifted. The procedure used was based on modifications of the method for particle size index for wheat hardness (AACC Method 55-30, 1995). Five grams of flour was sifted through a series of sieves (425, 250, 150, and 75 um) for 5 min. The sift and pulse amplitudes were set at "7" and "6", respectively. The resulting flour sample on each sieve was then weighed and recorded. A minimum flour recovery of 94% was attained. Duplicate analyses were conducted.

3.1.3.5 Colour of flours

The colour of micronized and non-micronized lentil seed and flour was measured using the Hunterlab MiniScan XE Colorimeter (Hunter Association Laboratory, Inc., Reston, VA). Sample colour was represented by L*, a*, and b* dimensions from the CIE Lab system. Illuminant A and a 10° observer were used. The instrument was standardized with a black and white tile (X = 79.1, Y = 83.8, Z = 89.4), with a pink tile (L = 76.2, a = 25.7, b = 17.4) scanned at regular intervals as a colour check.

Lentil samples were ground to pass through a 0.5-mm screen using a Cyclone Sample Mill (UDY Corporation, Fort Collins, CO). The seed or flour was then transferred to a petri dish (5 cm diameter) which was filled to the top. The dish was covered at the top with a similar sized glass dish. The sample was scanned under the Hunterlab port, rotated 90°, and re-scanned. The flour was then redistributed by shaking and re-scanned as before.

3.1.4 Functional properties of flours

3.1.4.1 Nitrogen solubility index (NSI)

NSI was determined using AACC Method 46-23 (1995) for nitrogen solubility and AACC Method 46-11 (1995) for Kjeldahl protein analysis. Two-hour extraction times were used. The pH of the samples was adjusted in 1-unit increments from pH 2 to 9 using 1.0 N HCl or NaOH.

3.1.4.2 Water holding capacity (WHC)

The WHCs of flours was determined using AACC Method 88-04 (AACC, 1995). A mixing time of 2 min was used instead of 1 min. Duplicate analyses were conducted.

3.1.4.3 Oil absorption capacity (OAC)

The OACs of flours were determined by the method of Ghavidel and Prakash (2006). A 1.0-g sample was mixed with 5 mL of corn oil in a 50-mL centrifuge tube for 1 min using a metal stirring rod, held for 30 min at room temperature, and centrifuged for 25 min at 5000 x *g* at 27 °C. The oil layer on the top of the sample was decanted and the tube was inverted for 5 min to drain residual oil, and then reweighed. Oil absorption capacities were calculated as grams of bound oil per gram of sample and expressed on a dry weight basis. Duplicate analyses were conducted.

3.1.4.4 Thermal properties of lentil

3.1.4.4.1 Differential scanning calorimetry (DSC)

The thermal denaturation properties of lentil flour were determined using a differential scanning calorimeter (Model Q2000, TA Instruments, New Castle, DE) calibrated with indium. First, 3.33 mg of flour (dwb) was weighed directly into an alodine-coated aluminum pan along with 10 µL of deionized water added to form a slurry of 75% moisture. The lid was crimped onto the pan to form a hermetic seal. Prepared samples were allowed to temper for 24 h at room temperature prior to DSC

analysis. The prepared DSC sample pan was subjected to a temperature increase from 40 to 140 °C at a ramp rate of 5 °C/min, with an empty pan used as reference. Onset (T_o) and peak (T_p) temperatures, and heat of enthalpy (ΔH) were measured from the thermograms generated using the Universal Analysis 2000 computer software Version 4.5A from TA instruments. All analyses were conducted in duplicate.

3.1.4.4.2 Pasting properties

The pasting properties of lentil flour were tested using a Rapid Visco Analyser (Newport Scientific Pty. Ltd., Warriewood, NSW, Australia). Three grams (dwb) of flour from non-micronized or micronized lentil seed was added to the canister containing 25.0 mL of deionized water. A plastic paddle was inserted into the canister which was jogged vertically 30 times, and then placed in the cavity of the RVA instrument. Standard Profile 2 was used according to AACC Method 76-21 (2000) where the suspension was equilibrated at 50 °C for 1 min, heated at 6 °C/min to 95 °C and held for 5 min, then cooled at 6 °C/min to 50 °C and held for 2 min. Continuous stirring at 160 rpm was maintained. All analyses were conducted in duplicate. Values (Cp) for peak viscosity, trough, breakdown, final viscosity, and setback, as well as peak time (min) and pasting temperature (°C) were obtained from the viscograms using Thermocline software from Newport Scientific Pty. Ltd.

3.1.5 Lipoxygenase activity of lentil flour

Lipoxygenase (LOX) activity was measured in lentil flour using the method outlined by McCurdy et al. (1983) with some modifications. A 1:10 lentil flour to buffer (0.05M, phosphate buffer, pH 6.9) ratio was mixed for 2 h at 4 °C to extract LOX. The slurry was then centrifuged for 30 min at 11,400 x *g* and filtered through Whatman #4 paper. A 0.1 mL aliquot of this crude extract was then transferred to a quartz cuvette containing 0.946 mM emulsified linoleic acid substrate, inverted three times within 5 sec, and read for 30 min at an absorbance of 234 nm using an Agilent 8453 Diode Array Spectrophotometer (Agilent Technologies Canada, Mississauga, ON). Absorbance data was recorded at 10 sec intervals using UV-Visible ChemStation Rev. A.08.03 software

(Agilent Technologies Canada, Mississauga, ON). Lipoxygenase activity was calculated from the slope of the linear region of the curve, which is approximately proportional to enzyme concentration, with 1 unit of lipoxygenase activity equivalent to an increase in absorbance of 0.001/min at 234 nm.

The substrate was prepared by roto-evaporating an emulsion of 2.72 mL each of 1% (w/v) linoleic acid in 95% (v/v) ethanol and 1% Tween 20 in 95% (v/v) ethanol and subsequently re-suspending with 50 mL of 0.05 M borate buffer (pH 9.0) and then adding 50 mL of 0.05 M phosphate buffer (pH 6.9) while using concentrated HCl to maintain pH 6.9. Substrate was used within 3 h. All solutions were stored at 4 °C under nitrogen gas until used.

3.1.6 Statistical analysis

The means and standard deviations of duplicate or triplicate determinations for the 11 flour samples (8 lentil types; 3 reference samples; soy, pea, and wheat flour) were calculated. The SAS Institute Inc. software (2008) was used to determine correlation coefficients between the different parameters ($P<0.05$).

3.2 Study II: Evaluating the effect of adding flours from micronized lentil to low-fat beef burgers

Burgers were produced with the addition of select flour binders that were characterized in Study I. Information on raw ingredients, manufacturing and cooking protocols, and analytical techniques used to assess burger quality are described.

3.2.1 Raw materials

All lentil (large green and red types) and wheat flours selected in this study were from the same batches as used in Study I. Toasted wheat crumb (enriched bleached wheat flour, niacin, iron, thiamine, mononitrate, riboflavin, folic acid, durum flour, leavening agent, dried yeast) was obtained from Newly Weds Foods (Edmonton, AB). Beef bottom rounds (Canadian AA or AAA Grade) were obtained from Centennial Foods Ltd., Saskatoon, SK. Meat for the three production reps were received and used within 9 to 24 days of animal slaughter. Incoming meat was stored at -1 °C until used. Salt (iodized) and pepper (32 mesh) were used as seasonings.

3.2.2 Burger formulations

The formulations of the low-fat beef burgers are summarized in Table 3.1. Burgers were formulated with various binders to replace meat, while keeping all other components constant. The flour from the large-sized red and green lentils (micronized or non-micronized) were used as binders at levels of 6 and 12%. Reference binders included a no-binder control, toasted wheat crumb, and wheat flour. All burgers were formulated to achieve 10% fat in the raw product.

Table 3.1: Low-fat beef burger formulations (10% fat) containing various binders at levels of 6 and 12%.

Burger Treatment	Ingredient					Total
(Binder Type)	**Beef[1]**	**Water**	**Salt**	**Pepper**	**Binder**	
Control						
0%	88.00	11.06	0.90	0.04	0.00	100.00
Green Lentil						
Non-micronized						
6%	82.00	11.06	0.90	0.04	6.00	100.00
12%	76.00	11.06	0.90	0.04	12.00	100.00
Micronized						
6%	82.00	11.06	0.90	0.04	6.00	100.00
12%	76.00	11.06	0.90	0.04	12.00	100.00
Red Lentil						
Non-micronized						
6%	82.00	11.06	0.90	0.04	6.00	100.00
12%	76.00	11.06	0.90	0.04	12.00	100.00
Micronized						
6%	82.00	11.06	0.90	0.04	6.00	100.00
12%	76.00	11.06	0.90	0.04	12.00	100.00
Toasted Wheat Crumb						
6%	82.00	11.06	0.90	0.04	6.00	100.00
Wheat Flour						
6%	82.00	11.06	0.90	0.04	6.00	100.00

[1]Meat from beef bottom rounds

3.2.3 Burger preparation

3.2.3.1 Manufacture of burgers

On the day before production, incoming meat was trimmed and separated into lean and fat component meat blocks. Any silver skin (connective tissue on the surface of the muscle) was removed and discarded. The lean and fat blocks were each ground through a 15.9-mm grinder plate (The Biro Manufacturing Co., Inc., Marblehead, OH). Representative samples obtained from the fat and lean components were analyzed for fat content using an HFT 2000 rapid fat analyzer (Data Support Co., Encino, CA). Meat blocks were covered and stored at -1 °C until ready for processing.

On the day of production, the Pearson square calculation was used to standardize the meat blocks so that a final raw burger fat content of 10% was achieved on a per batch formulation basis. The ground meat block and dry ingredients were transferred to a mixer bowl (Berkel BA-20 Mixer, Berkel Co., Countryside, IL) and cold water (0 °C) was slowly added while mixing for 45 sec. Each burger formula was then ground through a 4.8-mm grind plate. All processes were conducted below 4 °C. The batch order for processing was completely randomized and all equipment was rinsed with cold water between batches.

Burger mixtures were formed into 12-cm diameter burgers using a Hollymatic Patty Machine (Hollymatic Corporation, Countryside, IL) stocked with patty paper. Individual burgers were stacked and packaged into plastic-lined corrugated boxes that held 40 patties. Enclosed boxes were inverted and stored in a freezer at -30 °C. The meat temperature was monitored and recorded through any period of storage and after all grinding or mixing processes. Burger production was conducted in triplicate, with runs one week apart. Batches of burgers used for the consumer panel were prepared similarly. Representative samples from each batch were obtained, vacuum packaged, and stored at -20 °C for subsequent proximate analysis.

3.2.3.2 Cooking of burgers

Raw burgers were transferred to -20 °C frozen storage prior to cooking. At this time, the frozen burgers were separated and the patty paper removed. On the day of cooking, frozen burgers were removed from the freezer and loaded directly onto the conveyor belt (belt speed dial "10.5") of a preheated impingement oven at 190 °C (Lincoln, Fort Wayne, IN). As burgers approached the end of the conveyor belt, they were flipped once and allowed to cook for 1 min more. A temperature of 75 ± 1 °C at the geometric centre of the burger was attained for all treatments. The cooking times required to reach this temperature were 10 ± 1 min and 12 ± 0.5 min for burgers containing binder and no binder, respectively.

3.2.4 Proximate composition of raw and cooked burgers

Two raw and two cooked burgers from each treatment were vacuum packed and stored at -20 °C for proximate analysis. Moisture, protein, fat, and ash were measured by AOAC Method 950.46, 47.021, 960.39, and 920.153, respectively (1990). Meat samples were ground for 45 sec using a food processor at the highest speed (Braun, Procter & Gamble, Toronto, ON). All analyses were conducted in duplicate. Batches from the three replicates used for the trained sensory panel were analyzed, whereas batches from one replicate for the consumer panel were analyzed.

3.2.5 Cooking properties

Cooking yield and dimensional changes were measured on the burgers following cooking. Before cooking, frozen samples were weighed, and the diameter and thickness were measured. Frozen burgers were placed directly in the oven to cook. After cooking,burgers were cooled on a rack for 15 min, blotted once on each side with a paper towel, and re-weighed. The diameter and thickness were then measured for each sample. Calculations were as follows:

% cooking yield = (cooked burger weight)/(raw burger weight) x 100 %

$$\text{Change in burger diameter (\%)} = \frac{(\text{raw burger diameter} - \text{cooked burger diameter})}{(\text{raw burger diameter})} \times 100\ \%$$

$$\text{Change in burger thickness (\%)} = \frac{(\text{raw burger thickness} - \text{cooked burger thickness})}{(\text{raw burger thickness})} \times 100\ \%$$

Moisture and fat retention values of burgers after cooking, were calculated as follows:

$$\text{Moisture retention (\%)} = \frac{(\text{cooked weight} \times \%\ \text{moisture in cooked burger})}{(\text{raw weight} \times \%\ \text{moisture in raw burger})} \times 100\ \%$$

$$\text{Fat retention (\%)} = \frac{(\text{cooked weight} \times \%\ \text{fat in cooked burger})}{(\text{raw weight} \times \%\ \text{fat raw burger})} \times 100\ \%$$

3.2.6 Instrumental texture analysis

3.2.6.1 Shear force

Shear force analysis was conducted on four samples from each batch. Burgers were cooked according to Section 3.2.3.2, and allowed to cool for 1 h at room temperature before storing in plastic bags at 4 °C. The following day, a 2.5 cm x 5 cm wide strip was cut from each burger, ensuring that burger edges were excluded. Prepared samples were stored at 4 °C and when ready to test, were removed from the refrigerator and equilibrated to 20 °C. The texture press (TMS-Pro Texture Press, Sterling, VA) was equipped with a straight-edge blade fixture (31.8 mm thick, 7 cm

wide) and with a common base sample holder with a shear-slot that was 6.5 cm wide. Analysis was conducted using a TMS-Pro Texture Press (Food Technology Corp., Sterling, VA) interfaced with a computer using Texture Lab Pro Software, version 1.13-002. Analysis was conducted at two points on the sample using a crosshead speed of 250 mm/min. Shear force was expressed in Newtons (N).

3.2.6.2 Texture profile analysis

Texture profile analysis was conducted on samples cooked and prepared as described previously for shear force. For each treatment, eight cores (diameter = 2.54 cm) were removed from two burgers per production replicate. Analyses were conducted using a TMS-Pro Texture Press (Food Technology Corp., Sterling, VA) interfaced with a computer using Texture Lab Pro Software, version 1.13-002. Each sample was axially compressed to 50% of its original height at a crosshead speed of 100 mm/min. The following are parameters obtained from the output:

Hardness (N) = peak force during first compression cycle ("first bite")

Cohesiveness = ratio of peak force area during second compression to the peak force area during first compression (Area 2 / Area 1)

Springiness (mm) = height that food recovers during time elapsed between end of first bite and start of second bite

Gumminess (N) = product of hardness and cohesiveness

Chewiness (N mm) = product of hardness, cohesiveness, and springiness

3.2.7 Colour of raw and cooked burgers

3.2.7.1 Colour of raw burgers (fresh)

On each production day, two burgers from each treatment were placed individually on Styrofoam trays and overwrapped with oxygen permeable film. The film had a reported moisture vapour transmission rate of 33.9 g/100 in^2/24 h (Choice Wrap, Huntsman Packaging Co., Uniontown, OH). All samples were stored at 4 °C

under soft white light conditions (320 – 490 lux, Sylvania, Mississauga, ON). The sample position was rotated each test day to average variations in light exposure of individual burgers. Raw colour of burgers (CIE L* = lightness, a* = redness, b* = yellowness) on days 0, 1, 3, 5, and 7 was evaluated using a HunterLab MiniScan XE (Hunter Association Laboratory, Reston, VA). Samples were scanned twice, and rotating clockwise by a 90 ° between readings. Illuminant A and a 10 ° observer were used, with the instrument standardized with black and white tiles. MiniScan 45 computer software was used (Hunter Associaties Laboratory, Reston, VA).

3.2.7.2 Colour of cooked burgers

Eighteen days after burger production, burgers were cooked from frozen according to Section 3.2.3.2 and stored at 4 °C for 1 day prior to colour analysis. Each cooked burger was covered with plastic wrap (Huntsman Packaging Co., Uniontown, OH) and the surface was scanned using the Hunterlab Miniscan as described in Section 3.2.5.7.1. Subsequently, samples were cut horizontally with a non-serrated knife, and the interior was scanned immediately as described in Section 3.2.7.1.

3.2.8 Thiobarbituric acid reactive substances (TBARS) analysis

Raw burgers stored at -20 °C for 9 to 11 weeks were analyzed for TBARS in duplicate using modifications of the method of Bedinghaus and Ockerman (1995). The frozen burgers were ground for 60 sec in a Braun food processor (Procter & Gamble Inc., Toronto, ON) and a 5.0-g sample was transferred to a Stomacher sampling filter bag (95 µm thick, 17.5 cm x 30.0 cm; VWR Canlab, West Chester, PA). A 50-mL aliquot of extraction solution (20% w/v trichloroacetic acid (TCA) with 1.6% v/v phosphoric acid) was added to the sample bag and blending was continued for 2 min in a Stomacher Lab Blender (Model BA6021, Seward Limited, Edmunds, UK). Then, 50 mL of cold deionized water was added to the bag and blended again for 2 min. The slurry was transferred to two 100-mL Falcon centrifuge tubes (VWR Canlab, Mississauga, ON), and centrifuged for 20 min at 1000 x g. The supernatant was then filtered through a Whatman #1 filter paper into a 100-mL volumetric flask and made to volume using deionized water. A 5-mL aliquot of filtrate was transferred to a centrifuge tube and

mixed with 5.0 mL of 0.02 M thiobarbituric acid reagent. Sample tubes were placed in a boiling water bath for 35 min to facilitate formation of the pink-coloured thiobarbituric acid-malonaldehyde complex. After the solution was cooled for 10 min, absorbance was read at 532 nm using a Spectronic Genesys 5 Spectrophotometer (Spectronic Instruments, Inc., Rochester, NY).

A standard curve was created by mixing 1.5, 3.0, and 4.5 mL of 2 x 10^{-7} mol/ mL of 1,1,3,3-tetramethoxypropane (TMP) with 50 mL of the TCA extracting solution (cold); the volume was made up to 100 mL with deionized water. Five mL of this TMP/TCA mixture was mixed with 5.0 mL of TBA reagent, and the samples handled as described above. The standard curve consisted of absorbance versus TMP concentration.

Recovery of TMP used to spike a raw burger sample was determined. This percent recovery was used to calculate the TBARS value. Three 5.0-g raw beef burger samples were spiked with 1.5, 3.0, or 4.5 mL of TMP solution, and subsequently analyzed for TBARS as described above. The percent recovery of TMP was calculated as follows:

% Recovery of TMP = (Absorbance of spike on meat sample) / (Abs of TMP standard after dilution) x 100

To determine the K value;

K-value = (S/A) x MW x (107/SW) x (100/P)

Where,

S = standard concentration (moles/5 mL)
A = Abs (532 nm) of standard
MW = molecular weight of malonaldehyde (72.03 g/mol)
SW = sample mass (g)
P = percentage of TMP recovery
TBARS value = Abs of meat sample x K-value

3.2.9 Trained sensory analysis

Trained sensory (n = 13) and consumer (n = 107) panels were conducted on low-fat beef burgers containing various binders. Both studies were approved (#07-188) by the Behavioural Research Ethics Board Office at the University of Saskatchewan.

3.2.9.1 Burgers evaluated

Eleven burger formulations were subjected to evaluation by a trained sensory panel. The burgers were formulated to contain ≤10% fat, and were comprised of burgers containing eight lentil binders (two lentil types, micronized or non-micronized, at 2 different use-levels), two industry reference binders (toasted wheat crumb, wheat flour), and one no-binder control (Table 3.1). Trained panelists evaluated six samples on day 1 and another six different samples (five treatments plus one repeated control) on day 2. Three replications were conducted. For each replicate, all 10 binder treatments were completely randomized for the two days, with the no-binder control included on each day.

3.2.9.2 Recruitment and training of panelists

Panelists (ages 18 – 64) were recruited from the University community using advertisements. An on-line pre-screening questionnaire was issued to potential candidates to learn of any food restrictions, general interest in sensory analysis, and availability. Interested candidates provided written consent to participate in training.

Training consisted of familiarizing panelists to sensory definitions and protocols and took place during 14 sessions over a 3-month period. The panelists were trained to detect various ranges of burger juiciness, tenderness, saltiness, and flavour. In each training session, various burger samples were prepared and presented to demonstrate extreme ends of the sensory scale for each attribute. One attribute was featured for each session and the number of samples presented was gradually increased from three to six over time. Training samples were manipulated by changing fat and salt content, adding

pea flour, over-cooking, over-mixing of meat batches, or using a commercially manufactured beef burger to demonstrate the desired sensory effect.

3.2.9.3 Screening of panelists

During screening, six burger treatments were presented to panelists for evaluation on each of three separate occasions. The six samples were burgers containing no binder, 6% toasted wheat crumb, 6% wheat flour, 6% and 12% non-micronized green lentil, and high-fat (18%) burgers containing 6% micronized green lentil.

The results for each panelist were then analyzed by one-way analyses of variance (ANOVA) using the General Linear Models procedure of the SAS Institute Inc. (2008). The F-values generated by the panelists for each attribute were ranked to assess their ability to discriminate between treatments. The final panel consisted of 13 people; eight males and five females. A small snack or gift certificate was offered to panelists each time for their participation.

3.2.9.4 Trained sensory panel study

Trained panelists evaluated 11 different burger treatments in three replications which took place in six sessions over a 3-week period. The 13 final panelists were asked to score samples according to aspects of juiciness, texture, flavour, and acceptability. A total of 10 sensory characteristics (Appendix 1) were assessed using 6 or 8-point scales during evaluation of each burger treatment presented. Personal comments were encouraged and space was provided on the score card to document this information. Definitions of sensory terminologies were posted in each booth as a reference (Appendix 2).

3.2.9.5 Serving of burgers

At each panel session, panelists were served six different samples. Cooked burgers were cut into eight equal wedges, and each individual piece was trimmed to remove 5 mm of the outer edges. Two wedges from each burger were given to each

sensory panelist. Cooked samples were then placed in 3-random-digit coded ramekins and covered with an aluminum dish (VWR Canlab, Edmonton, AB) to minimize drying of samples. Prepared samples were then held in a 60 °C pre-heated incubator (Isotemp incubator, Fisher Scientific Ltd., Nepean, ON) for no more than 15 min prior to serving. When serving to panelists, prepared sample ramekins were removed from the incubator, and placed on aluminum foil-wrapped ceramic tiles that have been pre-heated in an oven (~90 °C). Panels were conducted three times per day starting 1 h apart, with panelists coming the same time each day. In total, two burgers from each treatment were cooked for each sensory session, where four to seven panelists would be served each time. The cooking order for all samples was completely randomized for each sensory day.

Samples were presented on a tray containing ambient temperature tap water and unsalted crackers for cleansing the palate between samples, toothpicks, spit cups, and appropriate score cards. The serving order of samples to each panelist was randomized to eliminate first-sample biases (Meilgaard et al., 2007).

The sensory room consisted of seven individual booths equipped with a swinging hatch door for delivering samples from the kitchen to each panelist. The room was dimly illuminated with red-filtered light to minimize the influence of sample colour biases during sensory evaluation. The room was regularly cleaned and air flow maintained to ensure a sanitary and odour-free environment.

3.2.10 Statistical analysis

The means and standard deviations of triplicate runs of the 11 burger treatments were calculated. Treatments were compared by a one-way analysis of variance (ANOVA) using the General Linear Models procedure of the SAS Institute Inc. (2008) to compare the effects of individual binder treatments on burger properties (proximate composition, cooking tests, texture profile, colour, TBARS). The level of significance was set at $p<0.05$. The least significant difference (LSD) procedure was used to compare individual treatment means. The main effects of binder colour or micronization were also assessed by partitioning the sums of squares due to lentil binder colour (red

versus green) and seed heat treatment (non-micronized versus micronized) into orthogonal contrasts ($p<0.05$).

Results for the 11 burger treatments from the trained sensory panel were compared using split plot analyses of variance (SAS Institute Inc., 2008). Burger treatment, replicate, and panelist, and their interactions were included in the model with treatment x replicate used as the valid error term for comparing treatments ($p<0.05$). The least significant difference (LSD) procedure was used to compare individual treatment means. Orthogonal contrasts ($p<0.05$) were used to compare the main effects of binder colour or micronization as previously described.

Pearson correlation coefficients (SAS Institute, 2008) were determined among the various parameters tested ($p< 0.05$).

3.3 Study III: Consumer panel study of low-fat beef burgers

Select treatments of low-fat beef burgers from Study II were assessed for their acceptability by a consumer sensory panel. The lentil binder chosen for this study was based on sensory results from Study II. Burger samples and manufacturing protocol, consumer panel recruitment, and sample scoring and consumer survey procedures are described.

3.3.1 Burger treatments

The four burgers tested contained: 1) 6% green lentil, non-micronized, 2) 6% green lentil, micronized, 3) 6% toasted wheat crumb, 4) no-binder control.

3.3.2 Recruitment of panelists

The consumer study was conducted on four days over a 3-week period with two to four panel sessions per day. A total of 107 consumers were recruited to participate in this study. The panel was comprised of students or employees of the University of Saskatchewan with demographics (sex, age) closely matching the statistical profile of the Canadian adult population (Statistics Canada, 2008), with the exception of larger and smaller proportions of the subjects from 18 to 29 years and 60 to 69 years, respectively (Figure 3.1). This was attributed to conducting the consumer panel on a university campus, where the age demographics leaned towards a younger population. A 1:1 gender ratio for each age group was targeted. Consumer recruitment was pre-arranged through the use of e-mail advertisements via offices of various departments, or conducted on-the-spot by intercepting potential participants at high-traffic locations on campus.

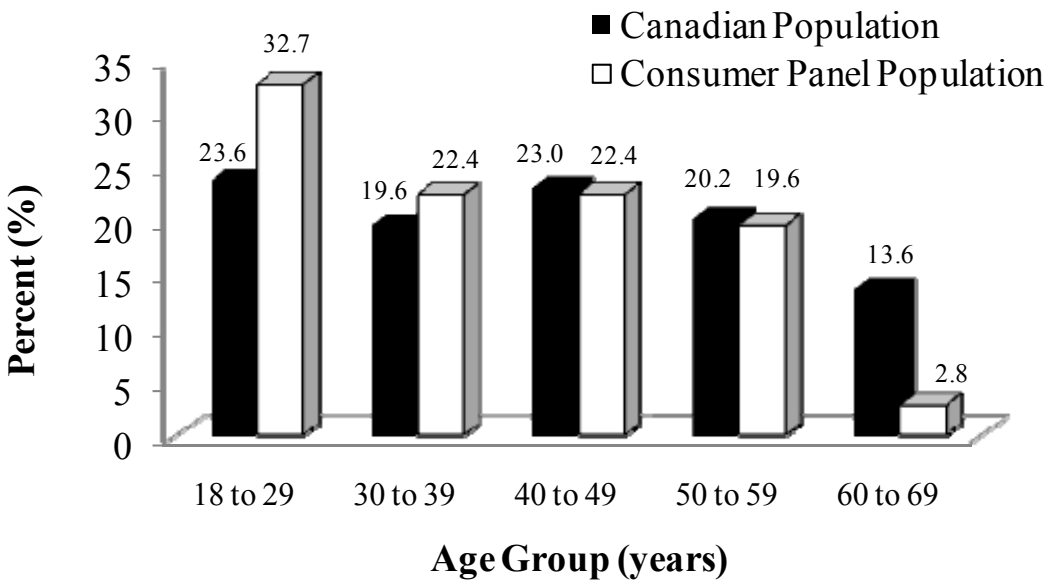

Figure 3.1: Age distribution of consumer panelist sample population (n=107) and the Canadian population. Canadian population data from Statistics Canada (2008).

3.3.3 Consumer study

Consumers were asked to come in one time for 15 min to complete two parts of this study. In Part I, panelists were asked to taste four samples and rate them using a simplified score card consisting of four attributes and their acceptability using 6-point hedonic scales. After tasting each sample, consumers were asked whether they would be willing to purchase the product (Appendix 3). Sample preparation and serving protocols were identical to that those described in Section 3.2.3. Procedures and vocabulary for tasting and describing beef burgers were provided (Appendix 4).

In Part II, panelists were asked to complete a 3-page consumer questionnaire (Appendix 5) which consisted of questions related to demographics and food purchasing and consumption behavior. Panelists were offered gift certificates for their participation.

3.3.4 Statistical analysis

Means and standard deviations were calculated for all treatments for the entire consumer sample population and for select segments. Treatments for the entire sample population and segments were compared by a one-way analysis of variance (ANOVA) using the General Linear Models procedure of the SAS Institute Inc. (2008). The least significant difference (LSD) procedure was used to compare individual treatments.

4. RESULTS AND DISCUSSION

4.1 Study I: Evaluating the effect of lentil type and heat treatment on the functional properties of lentil flour

4.1.1 Compositional and physical properties of flours

4.1.1.1 Moisture, fat, protein, ash content, and pH

The dehulled lentil seed used for this study was from four market classes (varying in seed colour and size) grown in Saskatchewan during the 2007 production year and were received from the supplier in 2008. Over a one-year period from the date of receiving, dehulled seeds were micronized and milled in preparation for subsequent testing as set out by the project objectives. Due to this extended lapse in time, the moisture contents of the four dehulled lentil types were analyzed twice, in 2008 upon receiving the seed, and again in 2009 when the majority of the analytical tests were conducted.

The proximate composition and pH values for four dehulled lentil types before and after micronization are displayed in Table 4.1 on a dry weight basis (dwb). The composition of the reference flours (pea, soy, and wheat) is also shown. Overall, proximate values for lentil from this study fell within the upper ranges presented in Wang and Daun (2006) who conducted analysis on non-dehulled Canadian green and red lentils. The fibre-rich hull comprises 7 to 8% of the lentil seed (Sokhansanj & Patil, 1995), and therefore removal of this fraction will generally raise the proximate values of other components in dehulled seed. There were no differences in pH, moisture, fat, protein, or ash due to lentil colour or size (Table 4.1)

Table 4.1: pH and proximate composition[2,3] (dwb) of lentil (dehulled), pea, soy, and wheat flours.

Sample	pH	Moisture 2008 (%)	Moisture 2009 (%)	Protein[1] (%)	Fat (%)	Ash (%)	Total Starch (%)	Gelatinized Starch[2] (%)
Green Lentil, Large								
non-micronized	6.4 ± 0.04	9.6 ± 0.03	8.6 ± 0.1	27.0 ± 0.5	0.9 ± 0.02	2.7 ± 0.04	-	-
micronized	6.5 ± 0.01	6.8 ± 0.03	5.7 ± 0.1	26.0 ± 0.2	1.2 ± 0.00	2.8 ± 0.04	46.0 ± 4.0	4.2 ± 1.0
Green Lentil, Small								
non-micronized	6.4 ± 0.00	8.8 ± 0.06	8.3 ± 0.1	27.8 ± 0.1	0.8 ± 0.01	2.6 ± 0.02	-	-
micronized	6.5 ± 0.02	6.5 ± 0.00	6.3 ± 0.1	27.3 ± 0.2	1.3 ± 0.03	2.6 ± 0.00	47.4 ± 2.2	5.0 ± 0.2
Red Lentil, Large								
non-micronized	6.4 ± 0.00	7.9 ± 0.07	5.5 ± 0.7	27.9 ± 0.0	1.6 ± 0.04	2.7 ± 0.02	-	-
micronized	6.5 ± 0.00	6.1 ± 0.04	5.8 ± 0.1	29.0 ± 0.0	1.9 ± 0.05	2.8 ± 0.01	46.0 ± 1.4	5.6 ± 0.8
Red Lentil, Small								
non-micronized	6.4 ± 0.01	9.1 ± 0.07	8.3 ± 0.0	29.0 ± 0.4	1.0 ± 0.02	2.6 ± 0.03	-	-
micronized	6.6 ± 0.01	5.4 ± 0.03	6.4 ± 0.0	28.9 ± 0.0	1.4 ± 0.01	2.6 ± 0.02	48.3 ± 0.7	2.5 ± 0.5
Pea Flour								
non-micronized	6.6 ± 0.00	-	10.3 ± 0.0	24.7 ± 0.8	1.4 ± 0.02	2.8 ± 0.01	-	-
Soy Flour								
non-micronized	6.2 ± 0.01	-	6.2 ± 0.0	52.6 ± 0.5	0.8 ± 0.03	7.0 ± 0.01	-	-
Toasted Wheat Crumb								
non-micronized	6.8 ± 0.01	-	9.1 ± 0.1	9.4 ± 0.1	0.4 ± 0.04	1.0 ± 0.03	-	-
Wheat Flour								
non-micronized	6.0 ± 0.01	-	9.0 ± 0.0	16.5 ± 0.2	1.0 ± 0.01	1.1 ± 0.01	-	-

[1]Protein was calculated as total nitrogen x 6.25 (legume) or x 5.7 (wheat)
[2]Values are means of two determinations ± standard deviation
[3] - not measured

Moisture content (2009) of all dehulled lentil samples ranged from 5.5 to 8.6% (Table 4.1). The native (non-micronized) large red lentil contained the least moisture (5.5%), compared with all other non-micronized lentils which contained 8.3 to 8.6% moisture. Seed intended for micronization was tempered by adding an amount of water appropriate to achieve the desitred seed moisture level (15%) prior to the heat treatment. After micronizing the seed to 135 °C surface temperature, the resulting moisture content ranged from 5.7 to 6.4% across all lentil types. These values are lower than those found in the literature where higher tempering moistures were used. In the study by Cenkowski and Sosulski (1997), large green lentils, tempered to 34% moisture prior to micronization for 120 sec to reach a final seed temperature of 140 °C, had a final moisture of 18%.

The moisture content of flour can also vary as a result of storage over time. Moisture changes were observed in lentil flour when measured upon receiving the raw material in 2008 and during the period of analysis of functional properties in 2009 (Table 4.1). Generally, the moisture levels of non-micronized and micronized lentil flours decreased over time, with the exception of micronized small red lentil flour which had the lowest moisture content (5.5%) initially upon receiving in 2008. Moisture values from 2009 were used to calculate proximate composition, as this period coincided with analytical testing.

The protein content of non-micronized lentil ranged from 27.0 to 29.0% (Table 4.1). These values are similar to those reported in the literature. Protein values for select Canadian lentil samples (non-dehulled) were reported to be 21 to 30% (Chung et al., 2008; Wang & Daun, 2006). Pea flour was lower in protein (24.7%) than the lentil flours, and defatted soy flour had the highest protein concentration (52.6%). Wheat flour contained 16.5% protein, whereas the toasted wheat crumb had an even lower amount (9.4%). The lower protein content found in toasted wheat crumb compared with the regular wheat flour could be due to the use of different wheat varieties or the level of refining of the flour.

The total fat content of dehulled lentils ranged from 0.9 to 1.6% (Table 4.1). Similarly, Wang & Daun (2006) observed 1.0 to 1.3% fat in lentils, and also found no differences among red and green lentils. Micronizing lentil appeared to increase the fat content to 1.2 to 1.9% (Table 4.1). The higher fat levels in micronized seed could be due to the heat-induced release of bound lipids, leading to a more efficient fat extraction during solvent reflux on the Goldfisch apparatus (Nielsen, 1998). In addition to this, the smaller particle size of micronized lentil flours may have increased the efficiency of crude fat extraction from the ground samples (Luthria et al., 2004). The observed fat content of lentil was similar in pea (1.4%), wheat flour (1.0%), and soy flour (0.8%), with the lowest content measured in toasted wheat crumb (0.4%).

The ash contents of dehulled lentil and pea flours ranged from 2.6 to 2.8% (Table 4.1). Soy flour had the highest ash content (7.0%), whereas wheat-based flours had the lowest (1.0 – 1.1%).

The pH of lentil flour slurries ranged from 6.4 to 6.6. There were no differences in pH among lentil types due to heat treatment. Pea flour had a pH (6.6) similar to that of lentil flour, whereas the soy and wheat flours ranged in pH from 6.0 to 6.2, and toasted wheat crumb had the highest pH at 6.8.

4.1.1.2 Total and gelatinized starch contents

Starch is a polysaccharide comprised of a large number of D-glucose units linked by α-(1,4) glycosidic bonds (amylose) or α-(1,4 and 1,6) glycosidic bonds (amylopectin). In the presence of heat and excess moisture, gelatinization of starch will occur where the granules swell resulting in the loss of crystalline structure (Nielsen, 1998). Changes in seed micro-structure on heating can impact starch functionality and is the basis of pre-cooked or par-boiled starch foods. Total and gelatinized starch contents of micronized lentil seed are presented in Table 4.1. The total starch content of dehulled lentil ranged from 46.0 to 48.3%, values slightly higher than those obtained by Chung et al. (2008) and Wang and Daun (2006) for green and red lentil varieties (non-dehulled). The latter study reported values of 43 to 46% total starch. As previously noted, the higher values

for total starch content in the current study were anticipated as the analysis was performed on dehulled seed.

The gelatinized starch content of micronized lentil ranged from 2.5 to 5.6% (Table 4.1). In comparison, Arntfield et al. (1997) found that increasing tempering levels (25, 29, and 33% moisture) increased starch gelatinization in lentil seed (~42, 52, and 70%), respectively, when micronized to a final seed moisture level of 12%. The degree of starch gelatinization reported by Arntfield et al. (1997) exceeds values obtained in the current study (15%) and can be attributed to differences in moisture levels attained during tempering. Arntfield et al. (1997) observed during pre-screening, that tempering seed to < 20% moisture prior to micronization was inadequate for achieving a desirable texture after micronized seed was conventionally cooked for 15 minutes. Sufficient moisture is required for starch swelling and leaching of amylose (Nielsen, 1998). Therefore, due to the low moisture (15%) targeted in the current study during seed tempering, the level of gelatinized starch achieved was minimal. Depending on the functional objective of micronization, the degree of gelatinization in lentil and other starch-rich seeds can be manipulated by controlling tempering conditions, or the micronizing rate or temperature.

4.1.2 Lipoxygenase activity

Lipoxygenase activity measured in dehulled lentil is presented in Table 4.2. Since lipoxygenase is known to be responsible for oxidizing polyunsaturated fatty acids to aldehydes and alcohols which contribute to off-flavours in legume seeds (Sessa, 1979), inactivation of this enzyme is critical for optimizing their shelf-life or usage in food products. Therefore, lipoxygenase activity was measured before and after micronization.

Table 4.2: Lipoxygenase activity[1] of lentil seed (enzyme units/g protein)

Sample	Lipoxygenase Activity (10^5 Units/g Protein)
Green Lentil, Large	
non-micronized	18.5 ± 0.8
micronized	0.1 ± 0.0
Green Lentil, Small	
non-micronized	19.7 ± 0.2
micronized	0.2 ± 0.0
Red Lentil, Large	
non-micronized	20.3 ± 1.6
micronized	0.2 ± 0.0
Red Lentil, Small	
non-micronized	20.1 ± 1.5
micronized	0.2 ± 0.0

[1]Values are means of duplicate determinations ± standard deviation

In general, untreated red lentil had slightly higher lipoxygenase activity (20.1 x 10^5 – 20.3 x 10^5 enzyme units/g protein) than did green lentil (18.5 x 10^5 – 19.7 x 10^5 units/ g protein). In comparison, Chang and McCurdy (1985) measured 24 x 10^9 enzyme units per gram protein extracted from native lentil cultivated in experimental plots. Values for lipoxygenase activity for other legumes include 28.9 x 10^9 units/gram protein for soybean (pH 9) and 12.6 x 10^9 units/gram protein for cowpea (Chang & McCurdy, 1985). Significant variations in reported lipoxygenase values exist and are commonly attributed to differences in quantitative techniques involving sample preparation, enzyme extraction conditions, substrate conditions, use of surfactant, and assay conditions (Chang & McCurdy, 1985). However, Al-Obaidy and Siddiqi (1981) reported that intact broad bean (with hull) had 50% more lipoxygenase activity than when de-hulled, which can help explain the lower values observed in the dehulled lentils in this experiment compared to values reported by Chang and McCurdy (1985). Moreover, significant variations in the lipoxygenase content of flaxseed can be attributed to cultivar, location, and seasonal conditions during cultivation (Oomah et al., 1997).

Overall, micronization of lentil to 135 °C reduced lipoxygenase activity in lentil 100-fold, from approximately 2,000,000 to 20,000 enzyme units/gram protein (Table 4.2). Another study investigating the effect of heat treatment on inactivation of lipoxygenase in legumes was that of Kouzeh-Kanani et al. (1982) where 95.5% of the lipoxygenase was inactivated within 60 sec of infrared treatment in soybeans.

4.1.2.1 Lentil colour

Seed from different lentil market classes have different cotyledon colours. Moreover, these colours may be affected when micronized and ground into flour. Results from HunterLab colour analysis of whole seed and flour from micronized and non-micronized lentils is shown in Table 4.3. The colour of whole red lentil was darker, more red, and more yellow (lower L*, and higher a* and b* CIE values) than that of green lentil. Upon grinding whole lentil seed (all green and red types), their respective flour colours became lighter, less red, and less yellow (higher L*, lower a* and b*). This is consistent with the concept that grinding plant materials decreases the colour of flour in proportion to its particle fineness (Cenkowski & Sosulski, 1997).

Table 4.3: HunterLab L, a, b colour values[1] for whole seed and ground flour from non-micronized and micronized lentil seed.

Whole Lentil Seed	**L***	**a***	**b***
Green Lentil, Large			
non-micronized	67.0 ± 0.3	14.2 ± 0.0	39.4 ± 0.3
micronized	64.3 ± 0.1	15.6 ± 0.1	50.0 ± 0.0
Green Lentil, Small			
non-micronized	68.5 ± 0.2	14.9 ± 0.1	45.1 ± 0.5
micronized	65.8 ± 0.7	15.3 ± 0.5	47.6 ± 1.4
Red Lentil, Large			
non-micronized	57.3 ± 0.3	34.1 ± 0.1	52.4 ± 0.5
micronized	59.1 ± 0.4	30.7 ± 0.6	50.8 ± 0.9
Red Lentil, Small			
non-micronized	57.1 ± 0.3	36.5 ± 0.3	55.4 ± 0.9
micronized	58.5 ± 0.3	30.3 ± 0.4	50.7 ± 0.3

Ground Lentil Flour	**L***	**a***	**b***
Green Lentil, Large			
non-micronized	89.4 ± 0.0	5.4 ± 0.0	26.7 ± 0.1
micronized	88.8 ± 0.2	4.9 ± 0.0	25.4 ± 0.1
Green Lentil, Small			
non-micronized	87.5 ± 0.1	5.8 ± 0.0	27.2 ± 0.1
micronized	85.4 ± 0.0	4.5 ± 0.0	23.1 ± 0.1
Red Lentil, Large			
non-micronized	84.4 ± 0.1	13.1 ± 0.1	25.3 ± 0.2
micronized	85.3 ± 0.1	11.6 ± 0.1	24.4 ± 0.1
Red Lentil, Small			
non-micronized	83.8 ± 0.1	16.3 ± 0.0	29.2 ± 0.1
micronized	86.8 ± 0.2	13.3 ± 0.1	26.8 ± 0.2
Toasted Wheat Crumb	80.1 ± 0.7	6.2 ± 0.0	22.0 ± 0.1
Wheat Flour	88.1 ± 0.2	4.6 ± 0.0	18.4 ± 0.1

[1]Values are means of duplicate determinations ± standard deviation
L* = lightness; a* = redness; b* = yellowness

Micronization influenced the colour of whole and ground lentil seed differently for green and red lentil. When micronized, whole seed of green lentil became darker, more red, and more yellow (lower L*, higher a* and b*), whereas red lentil seed became lighter, less red, and less yellow (higher L*, lower a* and b*). When micronized lentil seed was ground into flour, flours from both green and red lentil became less red and less yellow (lower a* and b*), while L* decreased for green lentil, and increased for red lentil. The wheat-based flours showed similar lightness and lower yellowness compared to all if the lentil flours, while the a* value (redness) was similar to that of the green lentil flours.

In comparison, the study of Arntfield et al. (2001) looked at micronizing non-dehulled green lentil to 138 and 170 °C which resulted in seed that was darker, more red, and less yellow as micronizing temperature increased. These trends are similar to the L* and a* values exhibited by green lentil in the current study, with the exception of yellowness (b*) which could be influenced by the grey colour of the hull that was left intact in Arntfield's study.

Loss of seed colour can also be associated with the destabilization of colour pigments when seeds were exposed to heat during storage. In the case of green lentil that had been exposed to 20 to 30 °C storage conditions over 3 weeks, destabilization of chlorophyll and loss of green colour in the cotyledon was noted (Nozzolillo & De Bezada, 1984). Moreover, pigment compounds found in red lentil contain polyphenolic structures. Amarowicz et al. (2009) identified quercetin diglycoside, catechins, digallate procyanidins, and *p*-hydroxybenzoic as the predominant polyphenolic compounds in red lentil that could also be influenced by extended ambient storage conditions.

The overall darkening effect observed in lentil as a result of micronization can also be attributed to Maillard browning, a reaction involving reducing sugars and amino groups of protein in the presence of low seed moisture (pre-tempered to 15%) and the use of dry infrared heat. Cenkowski and Sosulski (1997) found that lentil seed pre-tempered to lower moisture levels (19%) compared to 25% and 34% began to discolour the earliest when a seed temperature of 137 °C was reached after approximately 40 sec

of micronization. Moreover, the high content of starch and protein present in lentil seed will serve as reactants for the Maillard reaction. Despite the darkening effect, melanoidins produced from the Maillard reaction have metal chelating properties and, therefore, can contribute to elevated levels of antioxidant activities commonly found in heat-treated seed (Acar et al., 2009).

Notable are the higher standard deviations in the colour analysis of micronized whole lentil seed. This could be attributed to the heterogeneous absorption of infrared radiation into the seed during micronization. This was apparent in micronized red lentil, in which variable spotting colour effects were observed (Figure 4.1). This spotting could be due to inconsistent distribution of moisture in the seeds during tempering prior to micronization. Because the absorption of infrared radiation into a solid food is increased with higher moisture levels (Krishnamurthy et al., 2008), those surfaces imbibing more water will be most affected by the radiation, resulting in greater discolouration.

Figure 4.1: Images of non-micronized and micronized lentil seed.

4.1.2.2 Particle size distribution

Particle size distributions for flours from non-micronized and micronized lentil seed are presented in Figure 4.2. Among all non-treated lentil types, approximately 51% of the flour passed through the smallest sieve (< 75 µm), 56% was deposited on the 75 to 425 µm sieves, and 2.1 to 2.7% on the largest sieve size, > 425 µm.

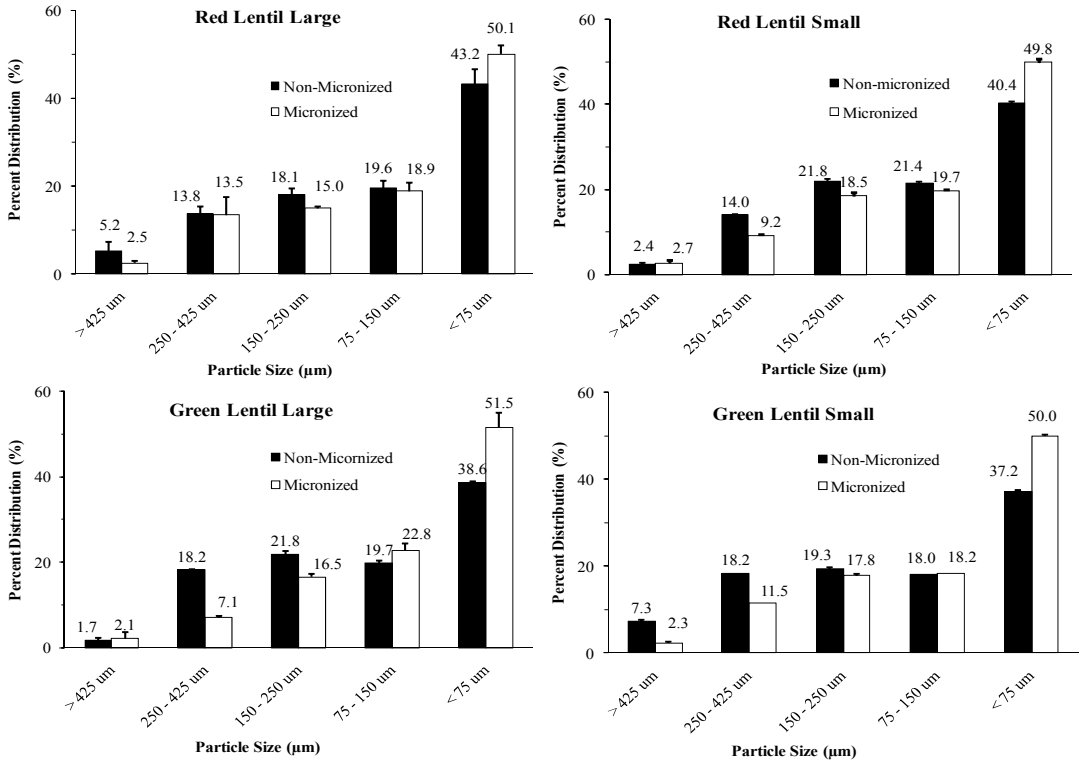

Figure 4.2: Particle size distributions of flour from non-micronized and micronized lentil seed. (Values are means of duplicate analysis).

For all lentil types, the effect of micronizing lentil seed is approximately 7 to 13% additional flour passing through the smallest-sized sieve, catching particles that were < 75 µm in size, at the expense of lower deposition on the larger-sized sieves. This higher proportion of flour of finer particle size was expected due to the effect that micronization has on the structural quality of the intact bean through starch gelatinization and simultaneous removal of moisture, as was demonstrated by Arntfield et al. (1997). Specifically, micronization can lead to a drier and more porous seed structure which in turn may increase the shattering effect of cyclone milling, resulting in a distribution favouring finer particle sizes.

4.1.3 Functional properties of lentil flour

Determining the functional properties of flour components in the lentil seed is important for understanding the possibilities for lentil applications. Nitrogen solubility is critical for proteins to exert functionality under specific conditions (pH), while water holding and fat binding capacities demonstrate the potential for a flour to contribute to moisture and fat retention in food products. In addition, monitoring how flours behave when exposed to controlled heat processes using viscometry and calorimetry can offer information about their stability at specific temperatures. These methods were used to detect functional differences among different seed types or as a result of seed micronization.

4.1.3.1 Nitrogen solubility index

The nitrogen solubility of lentil flour was measured in water as a function of pH and results are presented in Figures 4.3 and 4.4. Results for all lentil samples are consistent in showing that nitrogen solubility is greater at pHs below and above the minimum solubility point of pH 4 to 5. At the point of minimum solubility, all non-micronized green and red lentil samples exhibited 16 to 20% nitrogen solubility, which is in agreement with Carbonaro et al. (1997) who also observed a broad range of nitrogen solubility (15 – 25%) for lentil flour in water over the isoelectric pH range of 3.5 to 5.0. At an acidic pH of 2, nitrogen solubility ranged from 79 to 91%, whereas at an alkaline pH of 9, solubility ranged from 95 to 97%. At the lower and higher pH levels, proteins possess a net positive or negative charge, respectively, which can lead to greater particle repulsion and dispersion in an aqueous environment (Damodaran et al., 2008). Conversely, at the isolectric point, proteins exhibit a net zero surface charge such that decreased electrostatic repulsion or increased hydrophobic protein interaction will promote aggregation in an aqueous solution, thus leading to reduced solubility. This u-shaped trend in nitrogen solubility of native lentil protein under aqueous conditions (pH 1 to 12) was also observed by Carbonaro et al. (1997), Bora (2002), and Ghavidel and Prakash (2006).

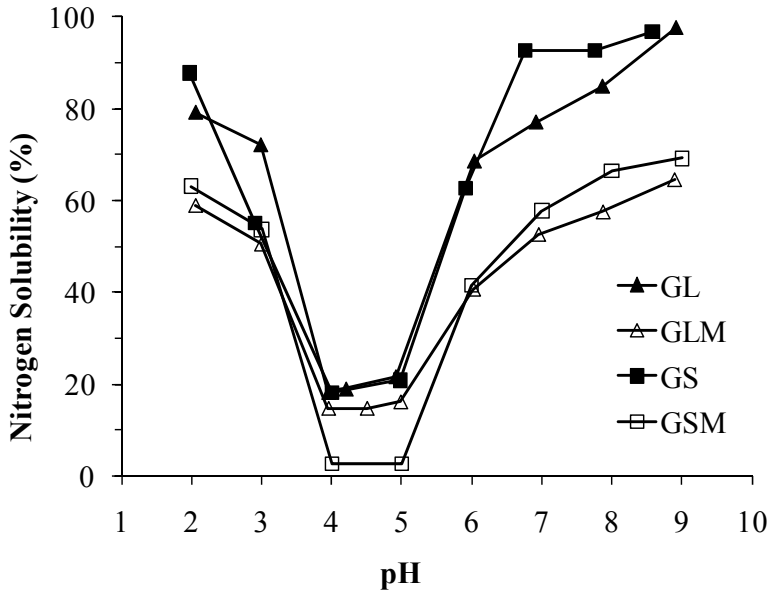

Figure 4.3: Nitrogen solubility curves for lentil flours from non-micronized and micronized green lentil seeds (large and small) over pH 2 – 9. GL = non-micronized large green lentil; GLM = micronized large green lentil; GS = non-micronized small green lentil; GSM = micronized small green lentil.

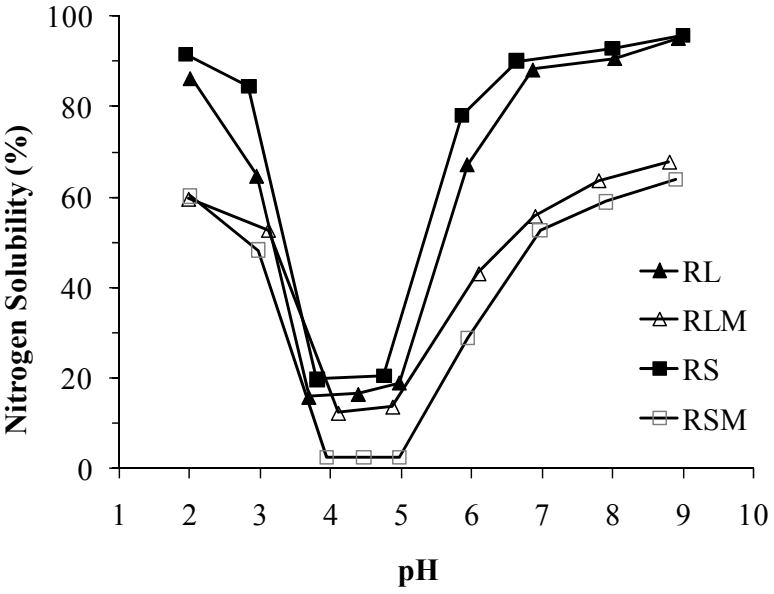

Figure 4.4: Nitrogen solubility curves for lentil flours from non-micronized and micronized red lentil seeds (large and small) over pH 2 – 9. RL = non-micronized large red lentil; RLM = micronized large red lentil; RS = non-micronized small red lentil; RSM = micronized small red lentil.

Overall, micronization of all dehulled lentil types decreased the nitrogen solubility of the flour over the pH range of 2 to 9 (Figures 4.3 and 4.4). This reduced solubility could be due to the heat-induced denaturation of proteins, where the unfolding of these structures exposes hydrophobic amino acid residues at the protein surface leading to aggregation in aqueous solution (Damodaran et al., 2008). Zheng et al. (1998) demonstrated through testing of nitrogen solubility on the residue from Osborne fractionation in sodium dodecyl sulphate (SDS) and 2-mercaptoethanol (MCE) buffer solutions, that the aggregation of proteins from micronized lentils was more likely due to hydrophobic aggregation as opposed to the formation of intermolecular disulfide bonds.

The degree of nitrogen solubility observed in the flours from heat-treated lentil varied depending on the lentil type and the pH of the prepared slurry. For example, at pH 6, there was a 33 - 64% loss in solubility with micronization for all green and red lentil types (Figures 4.3 and 4.4). Near the isoelectric point (pH 4 – 5), small lentil varieties exhibited a greater reduction in nitrogen solubility upon micronization (85 – 89%) than did the large lentil varieties, which exhibited a smaller reduction (20 – 23%). The greater reduction in solubility displayed by the smaller sized lentils indicates that the proteins from these smaller-sized lentils had experienced a greater degree of denaturation and that hydrophobic forces had outweighed electrostatic forces at the protein/peptide surface. This greater potential for denaturation could be due to the smaller size of the lentil seed which allowed for a deeper penetration of radiation or conductive heating into the seed during micronization compared with the larger-sized seed.

Similar reductions in solubility as a result of micronizing lentil were found in the literature. Zheng et al. (1998) observed a 31% decrease in nitrogen solubility at pH 6 upon micronization of lentil (8% moisture) to 140 °C, and Nagmani and Prakash (1997) observed a 34% decrease in nitrogen solubility at pH 6 upon exposure of dehulled lentil seed to dry heat in a pressure cooker for 10 min at 15 psi. However, at pH 4, the former authors found no changes in lentil nitrogen solubility regardless if the samples were heat-treated, while the latter authors observed a 50% decrease in solubility at pH 4,

which corresponded with the current study. This could be due to the absence of tempering of seeds in Zheng et al. (1998) prior to micronization, as they indicated that the starting moisture was 8%. Their study also showed that increasing seed moisture content and micronization temperature progressively reduced nitrogen solubility in cereals, even at pH 4.

4.1.3.2 Water holding capacity and oil absorption capacity

The water holding capacity (WHC) and oil absorption capacity (OAC) are important properties to consider for food ingredients as they are relevant to enhancing the sensory quality of the food product, particularly those low in fat. The WHC and OAC of lentil, pea, soy, and toasted wheat crumb are shown in Table 4.4. Lentil colour and size did not have a significant impact on WHC. Defatted soy flour displayed the greatest WHC (2.3 g/g), and pea and wheat flour showed values similar to those of the untreated lentil samples. The WHC of micronized lentil was about 27% higher (1.0 – 1.1 g/g) than that of non-micronized lentil (0.7 – 0.8 g/g). Similarly, in Fasina et al. (2001), the WHC of lentil flour increased by approximately 25% as a result of micronizing seed to 140 °C.

The higher level of protein in defatted soy flour (52.6%) compared with lentil (26 – 29%), pea (24.7%), or wheat (16.5%) could explain the higher WHC. Rao et al. (2002) obtained a similar WHC value for native defatted soy flour (2.35 g/g). Also, Sosulski and McCurdy (1987) measured the WHC of soy flour (48% protein) to be 1.75 g/g, which was increased to 2.65 g/g when the protein was further purified to 82% protein. Generally for proteins, the greater the number of charged residues present, the greater the potential hydration capacity. In the current study, nitrogen solubility at a pH of approximately 6.5 was higher than at the isoelectric pH of 4.5 (Figures 4.3 and 4.4). This aqueous environment at a pH above the isoelectric point would result in de-protonation of amino groups at equilibrium leading to a net negative protein surface charge and increased hydrophilicity (Damodaran et al., 2008).

Table 4.4: Water holding and oil absorption capacities[1] of lentil, pea, soy, and wheat flours.

Sample	WHC (g/g)	OAC (g/g)
Green Lentil, Large		
non-micronized	0.7 ± 0.03	0.7 ± 0.03
micronized	1.0 ± 0.05	0.8 ± 0.04
Green Lentil, Small		
non-micronized	0.8 ± 0.02	0.8 ± 0.00
micronized	1.1 ± 0.05	0.8 ± 0.04
Red Lentil, Large		
non-micronized	0.8 ± 0.07	0.8 ± 0.05
micronized	1.0 ± 0.01	0.8 ± 0.04
Red Lentil, Small		
non-micronized	0.8 ± 0.05	0.7 ± 0.03
micronized	1.0 ± 0.03	0.8 ± 0.03
Pea Flour		
non-micronized	0.9 ± 0.00	1.1 ± 0.09
Soy Flour		
non-micronized	2.3 ± 0.00	1.1 ± 0.01
Wheat Flour		
non-micronized	0.8 ± 0.06	1.0 ± 0.02

[1]Values are means of duplicate determinations ± standard deviation and are expressed on a dry weight basis.

The higher WHC observed in flours from micronized lentils compared with those from non-micronized lentil could be due to the increased water binding of the exposed amylose/amylopectin or amino acid residues that resulted from the heat-induced partial starch gelatinization and protein denaturation, respectively. Increased hydration contributed by starch can occur as migration of water molecules into the starch granule creates hydration layers around amylose and amylopectin, which leads to swelling. Furthermore, when lentil seed was micronized to 135 °C, heat-induced unfolding of proteins was likely to have occurred, exposing even more charged surface residues and effectively enhancing the WHC of the flour. Similar thermally-induced (dry or moist heat sources) increases in WHC were observed in pea flour (Megha & Grant, 1986), in lentil, green, black, and Bengal gram (Nagmani & Prakash, 1997), and in lentil, pea, and various beans (Fasina et al., 2001).

The OAC did not differ between lentil flour types or micronization treatments. The pea, soy, and wheat flours exhibited higher OAC (1.0 – 1.1 g/g) than did lentils (0.7 – 0.8 g/g) (Table 4.4). Similar trends as a result of micronization were observed for cowpea flour by Mwangwela et al. (2007). This lack of difference between micronized and non-micronized lentil samples could be attributed to insufficient hydrophobic or non-polar residue exposure resulting from the degree of protein denaturation achieved.

4.1.3.3 Thermal properties

4.1.3.3.1 Differential scanning calorimetry

Differential scanning calorimetry (DSC) analysis was conducted on lentil flours at a 5 °C/min ramp rate and the resulting thermograms are shown in Figure 4.5. The parameters (T_o, T_p, ΔH) obtained from integrating the thermal events are shown in Table 4.5.

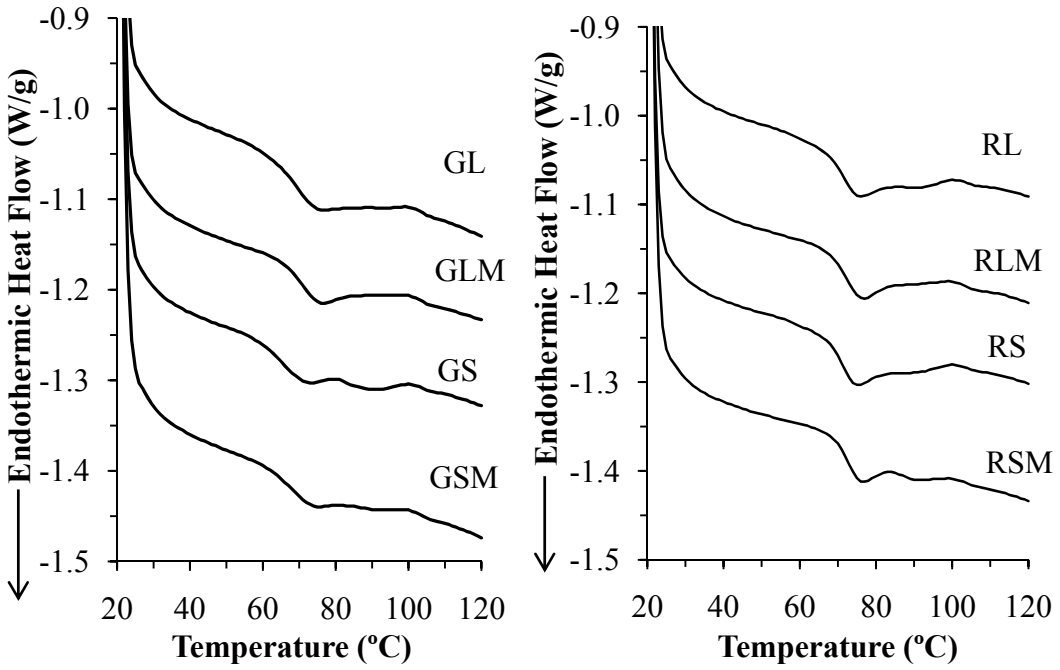

Figure 4.5: DSC thermograms for flours from non-micronized and micronized lentil seed (GL = non-micronized large green lentil; GLM = micronized large green lentil; GS = non-micronized small green lentil; GSM = micronized small green lentil; RL = non-micronized large red lentil; RLM = micronized large red lentil; RS = non-micronized small red lentil; RSM = micronized small red lentil).

Table 4.5: Differential scanning calorimetry properties[1] of flours from non-micronized and micronized lentil seed (25% lentil slurry, ramp rate = 5 °C/min)

Sample	T_o (°C)[2]	T_p (°C)[2]	ΔH (J/g)[3]
Green Lentil, Large			
non-micronized	62.9 ± 0.04	74.9 ± 0.01	9.8 ± 0.05
micronized	66.6 ± 0.40	75.8 ± 0.25	5.8 ± 1.06
Green Lentil, Small			
non-micronized	58.5 ± 1.04	71.7 ± 0.31	9.5 ± 0.30
micronized	61.3 ± 0.13	73.7 ± 0.04	7.4 ± 0.25
Red Lentil, Large			
non-micronized	67.1 ± 0.12	75.6 ± 0.03	9.6 ± 0.01
micronized	67.9 ± 0.11	76.3 ± 0.16	8.3 ± 0.10
Red Lentil, Small			
non-micronized	67.3 ± 0.14	75.1 ± 0.02	9.8 ± 0.20
micronized	69.2 ± 0.33	76.0 ± 0.29	7.1 ± 0.17

[1]Values are means of duplicate determinations ± standard deviation
[2]T_o = onset temperature; T_p = peak temperature; ΔH = heat of enthalpy
[3]Data expressed on a dry weight basis

The DSC thermograms of the non-micronized lentil flours exhibited one broad endothermic event (Fig. 4.5) with an onset temperature (T_o) of 57 to 62 °C, and a thermal peak (T_p) ranging from 71.7 to 75.1 °C. There were some differences in thermal behavior (T_p) among lentil types; thermograms for small green lentils showed a peak at the lowest temperature (71.7 °C) while thermal peaks for the other lentil types ranged between 74.9 and 75.6 °C. These transitions are likely related to starch gelatinization. Native lentil starch extracts documented in the literature have been shown to exhibit thermal peaks characterized by onset gelatinization temperatures (T_o) of 58 to 63 °C and peak gelatinization temperatures (T_p) of 67 to 70 °C (Chung et al., 2009; Sandhu & Lim, 2008; Sosulski et al., 1985).

When micronized, T_o and T_p of the flours were delayed by 1 to 4 °C and 1 to 2 °C, respectively, and the corresponding enthalpy (ΔH) decreased by 13 to 40% (Table 4.5). A 4 to 5 °C delay in T_p and T_o was observed by Mwangwela et al. (2007) in their study of micronized cowpea to a surface temperature of 130 °C. This delay in the occurrence of thermal events exhibited by micronized samples implies that a higher

temperature is required for gelatinization and could be attributed to the stabilization of the partially gelatinized or denatured flour components. Moreoever, Mwangwela et al. (2007) observed enthalpy decreases of 12 to 28% upon micronizing cowpeas up to 170 °C. It was suggested that these smaller enthalpies as a result of micronization in non-fractionated flours were due to starch or protein gelatinization or denaturation, respectively.

DSC thermograms did not display resolved thermal events indicative of protein denaturation, despite lentil flour containing up to 29% protein. Various lentil protein extracts have been shown to exhibit thermal peaks at 92 °C (alkaline extracted; Lee et al., 2007), and at 99 °C (air classified; Sosulski et al., 1985). In the current study, the lack of a DSC thermal peak at this temperature range could be due to the heterogeneous nature of the flour. Because the presence of starch and protein in the lentil flour are expected to yield thermal events occurring within approximately 30 °C of each other, overlapping of the end and beginning of the two thermal events can result in one overall broad peak. Broad peaks were observed in the current study and are characterized by ΔH values (9.5 – 9.8 J/g). In comparison, Chung et al. (2008) observed a distinct peak occurring at 92 to 95 °C in addition to a peak attributed to gelatinization at 76 °C. Higher volumes and concentrations of flour slurries prepared in the DSC pans or slower ramp rates (Nielsen, 1998) in general could improve the resolution of the thermal events caused by starch and protein. In the study by Chung et al. (2008), 12 mg of non-dehulled lentil flour was mixed with 28 μL of water (30%), whereas in the current study, 3.3 mg of lentil flour was mixed with 10 μL of water (25%).

4.1.3.3.2 Pasting properties

The pasting properties of lentil flour are presented in Table 4.6 and Figure 4.6. As temperature was increased from 50 to 95 °C and held at 95 °C, the viscosity of the lentil slurries increased dramatically and approached a peak viscosity ranging between 711 to 996 cP occurring at 12.9 to 13.0 min of the heating program.

Table 4.6: Pasting characteristics[1] of non micronized and micronized lentil.

Sample	Peak Viscosity (cP)	Breakdown (cP)	Peak Time (min)	Pasting Temp (°C)
Green Lentil, Large				
non-micronized	996 ± 6.4	1 ± 3.5	12.9 ± 0.1	77.3 ± 0.2
micronized	1241 ± 6.4	95 ± 2.8	9.3 ± 0.2	76.4 ± 0.0
Green Lentil, Small				
non-micronized	711 ± 7.8	-1 ± 1.4	13.0 ± 0.0	78.3 ± 0.1
micronized	1100 ± 36.8	61 ± 5.7	10.0 ± 0.1	75.8 ± 0.3
Red Lentil, Large				
non-micronized	716 ± 7.1	-1 ± 1.4	13.0 ± 0.0	78.7 ± 0.0
micronized	1007 ± 19.1	28 ± 5.7	10.3 ± 0.1	77.4 ± 0.3
Red Lentil, Small				
non-micronized	776 ± 16.3	-3 ± 1.4	12.9 ± 0.0	78.1 ± 0.1
micronized	942 ± 36.8	36 ± 5.7	10.1 ± 0.1	77.3 ± 0.3

[1]Values are means of at least two determinations ± standard deviation
cP = centipoises

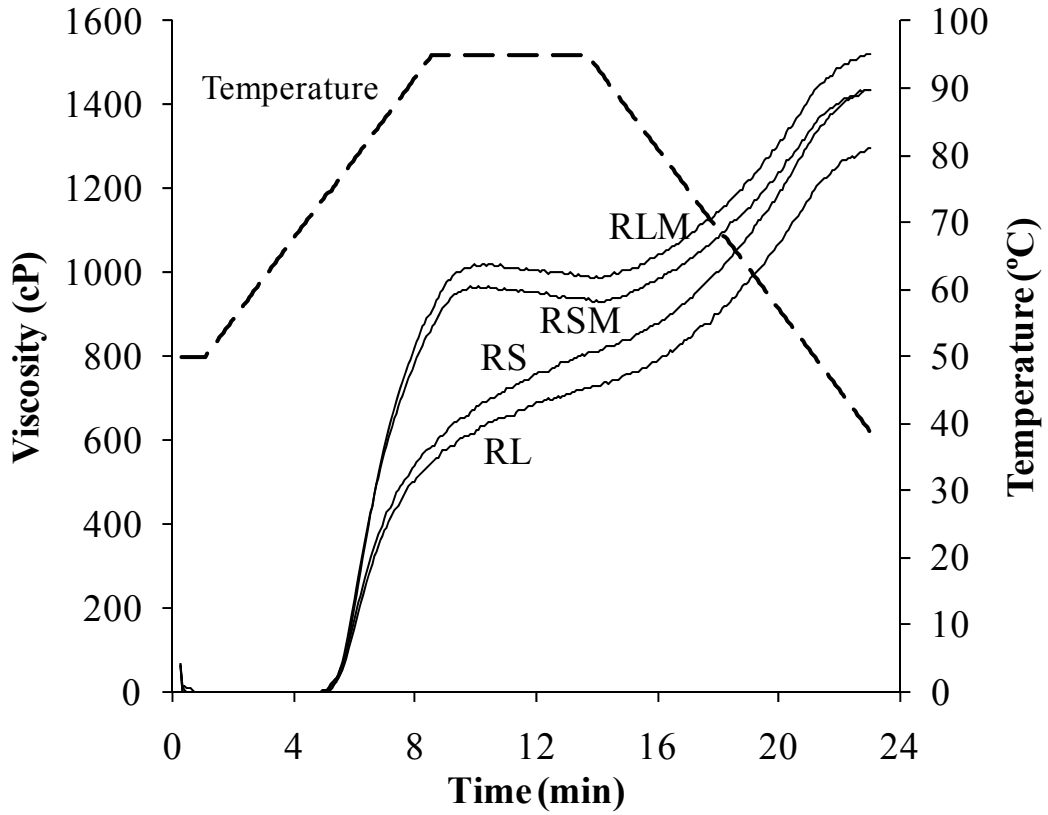

Figure 4.6: Viscograms for red lentil flours. RL = non-micronized large red lentil; RLM = micronized large red lentil; RS = non-micronized small red lentil; RSM = micronized small red lentil.

The temperature at which the slurry started to paste was 77.3 to 78.7 °C. This pasting behaviour can be explained by the water entering the starch granule and hydrating the amylose and amylopectin molecules, leading to granule swelling and initiating the breakdown of the granule, or gelatinization. When measuring the pasting properties of flour from micronized lentil, peak viscosities increased by 21 to 55% to 942 to 1241 cP while the time to reach these respective peaks decreased by 2.7 to 3.6 min. Moreover, the temperature at which the slurry began to paste decreased as a result of micronization. Peak viscosity is indicative of the water-binding capacity of the sample, and pasting temperature can be indicative of minimum cooking temperatures (Newport Scientific Pty. Ltd., 1998). Therefore, micronization of lentil seed (15% moisture, 135 °C) can increase the water binding capacity and lower cooking times by initiating gelatinization. The non-micronized samples would gelatinize under the RVA test, while the proteins would stay intact under this RVA temperature program which applies a maximum holding temperature of 95 °C. Because lentil proteins have been shown to denature at approximately 92 °C (Lee et al., 2007), the maximum holding temperature attained is likely insufficient to denature the protein.

Variations in the effects of micronization of legume seeds on peak viscosity are found in the literature. Mwangwela et al. (2007) reported peak viscosities contrary to the current study where they observed increasing micronization temperatures (41% moisture, 130 and 170 °C) resulted in decreased peak viscosities for a cowpea flour slurry compared to that of a non-micronized control. Conversely, the pasting results of Cenkowski & Sosulski (1998) in their study of micronized pea (26% moisture, 500 W IR exposure for 90 seconds) was more consistent with the current study where micronization induced a 21 to 55% elevation in peak viscosity. The reason that Mwangwela et al. (2007) found a lower peak viscosity as a result of micronization may be due to the extreme micronization conditions employed (41% moisture, 170 °C), which would have resulted in severe degradation of starch granules leading to lower viscosities.

4.1.3.4 Correlation coefficients for lentil flour properties

There was a significant ($p<0.001$) negative correlation between peak viscosity and pasting temperature (-0.91), peak time ($r = -0.83$), and heat of enthalpy (-0.78) (Table not shown). As peak viscosity became greater, the pasting temperature was lower, the time to reach this peak decreased, and the heat enthalpy was reduced. At the peak viscosity, or the point before granule leaching, WHC of the starch molecules in the granule is expected to be the greatest. Although positive, this correlation was relatively low between the peak viscosity and WHC ($r = 0.64$, $p<0.01$) indicating that there may be variables other than those relating to water holding that may have contributed to the increased peak viscosities.

There was a significant ($p<0.001$) correlation between WHC and heat of enthalpy ($r = -0.87$,), peak time ($r = -0.93$), and pH ($r = 0.80$). When lentil displayed greater WHC, samples also required lower heat energy for their thermal transitions to occur or peaked earlier, likely because the starch was already partially gelatinized. The positive correlation between pH and WHC indicate that samples in higher pH conditions will yield greater WHC. This is expected since proteins became more charged at pHs above the isolelectric point, offering greater interaction with water.

4.1.4 Summary

Dehulled seed from four lentil market classes (seed colour and size) was tempered (15% moisture), micronized (135 °C), and ground into flour. Each seed type was analyzed for its proximate composition, degree of gelatinization, lipoxygenase activity, colour, nitrogen solubility, water holding and oil absorption capacities, and thermal characteristics. Results were compared with those of non-micronized lentils, defatted soy, and wheat-based reference flours.

Dehulled lentil is high in protein and starch, and low in fat. The proximate composition was similar among lentil types. The amount of starch gelatinized (2.5 – 5.6%) as a result of micronization was minimal compared with other studies due to the relatively low tempering moisture and micronization temperature employed.

Nonetheless, this level of gelatinization was able to reduce pasting temperature by 1 to 3%, indicating that some of the starch had been pre-gelatinizated due to the treatment. To further support this, a 13 to 40% decrease in ΔH was observed from DSC analysis, signifying the occurrence of thermal impacts on the lentil components.

Some level of protein denaturation as a result of micronizing lentil was evident from the nitrogen solubility tests over the pH range of 2 to 9, which showed reduced solubility to a variable degree depending on pH. At pH 6, a 33 to 64% loss in nitrogen solubility with micronization was demonstrated for all lentil samples, representing partial protein denaturation. This loss of nitrogen solubility could be necessary for attaining the 25 to 43% increase in WHC observed in flour from micronized lentil. The increase in WHC was presumably contributed by the partial unfolding of proteins, exposing their charged side chains. There were no changes in OAC. Moreover, the micronization of lentil resulted in a 100-fold reduction in lipoxygenase activity, suggesting that the heat of micronization sufficiently impacted the protein structures of the enzymes, causing inactivity.

Micronization also affected physical properties of lentil such as seed colour and grinding behavior. Micronization of seed caused green and red lentils to lose their green and red colour, respectively. Seed discolouration could be desirable in terms of minimizing colour carry-over into food applications requiring a neutral colour. The particle size distribution favoured additional deposition of flour (7 – 13%) on the smaller-sized sieves.

The development of low-fat meat products can be enhanced with the use of binders that may offer increased functionality such as water holding capacity. The effect of lentil size did not show any dramatic differences among their proximate values, functional properties, and thermal behaviour. Since the red and green lentil seeds were significantly different in colour, flours from their large lentil types were chosen to be used as binders in the development of a low-fat beef burger. Moreover, the supply of large lentil types within the green and red lentil market classes was generally more readily available.

4.2 Study II: Evaluating the effect of adding flours from micronized lentil to low-fat beef burgers

4.2.1 Proximate composition and pH of raw and cooked burgers

Low-fat beef burgers were manufactured with the addition of flour from non-micronized and micronized lentil seed from the large green and large red market classes evaluated in Part I. The moisture, protein, fat, and ash contents, and pHs of raw and cooked low-fat burgers containing different binders are presented in Table 4.7 and 4.8.

4.2.1.1 Raw burgers

The composition of raw beef burgers ranged from pH 5.5 to 5.7, 63.4 to 72.1% moisture, 16.6 to 17.9% protein, 8.5 to 9.5% fat, and 1.7 to 1.9% ash (Table 4.7). There were no significant differences ($p<0.05$) in fat content among the burger treatments. However, values ranged between 8.5 to 9.5%, which falls below the 10% fat level targeted. Factors such as the sampling of heterogeneous meat or the measuring of fat levels in the fat and meat blocks during manufacture by using a rapid fat method could account for this discrepancy. The fat content determined using the HFT 2000 rapid fat analyzer is based on an internal fat program model. This model is an indirect measure of fat content which correlates the moisture lost during controlled heating with the fat content in beef.

The moisture content was highest for the control burger (72.1%), and was significantly lower ($p<0.05$) for burgers containing 6% lentil flour (67.5 – 67.7%) and even lower for those with 12% lentil flour (63.4 – 64.2%) (Table 4.7). As higher binder levels were used, more meat was displaced in the burger formula. With beef containing approximately 70% water (Wahrmund-Wyle et al., 2000) compared with <10% moisture in lentil, burgers containing more binder will have in lower moisture contents.

Table 4.7: Proximate composition[1,2] and pH[1,2] of raw low-fat beef burgers formulated with various lentil flours and wheat binders at addition levels of 6 or 12% (w/w).

Binder Level	pH	Moisture	Protein (%)	Crude Fat	Ash (%)
Control					
0%	5.5 ± 0.06 ^{e}	72.1 ± 1.6 ^{a}	17.5 ± 0.8 ^{ab}	8.9 ± 2.5 ^{a}	1.7 ± 0.07 ^{d}
Green Lentil					
Non-micronized					
6%	5.5 ± 0.04 ^{cd}	67.6 ± 1.4 ^{b}	17.4 ± 0.6 ^{ab}	8.9 ± 1.6 ^{a}	1.8 ± 0.05 ^{cd}
12%	5.6 ± 0.08 ^{abc}	64.2 ± 1.1 ^{c}	17.5 ± 0.8 ^{ab}	9.1 ± 1.5 ^{a}	1.8 ± 0.01 ^{bc}
Micronized					
6%	5.6 ± 0.04 ^{cd}	67.5 ± 0.9 ^{b}	17.2 ± 0.8 ^{bc}	8.8 ± 1.5 ^{a}	1.8 ± 0.05 ^{cd}
12%	5.6 ± 0.03 ^{bc}	64.1 ± 0.8 ^{c}	17.9 ± 0.2 ^{a}	8.5 ± 1.3 ^{a}	1.9 ± 0.02 ^{a}
Red Lentil					
Non-micronized					
6%	5.6 ± 0.01 ^{bc}	68.2 ± 1.2 ^{b}	17.5 ± 1.0 ^{ab}	8.5 ± 2.1 ^{a}	1.8 ± 0.06 ^{cd}
12%	5.7 ± 0.06 ^{a}	63.8 ± 0.9 ^{c}	17.9 ± 1.0 ^{a}	8.8 ± 1.0 ^{a}	1.9 ± 0.03 ^{ab}
Micronized					
6%	5.6 ± 0.06 ^{cd}	67.7 ± 0.8 ^{b}	17.4 ± 1.1 ^{ab}	9.5 ± 1.4 ^{a}	1.8 ± 0.01 ^{bc}
12%	5.7 ± 0.07 ^{ab}	63.4 ± 1.3 ^{c}	17.8 ± 0.7 ^{a}	9.4 ± 1.6 ^{a}	1.9 ± 0.02 ^{a}
Toasted Wheat Crumb					
6%	5.5 ± 0.01 ^{de}	68.3 ± 1.4 ^{b}	16.6 ± 0.9 ^{d}	8.5 ± 1.8 ^{a}	1.7 ± 0.05 ^{d}
Wheat Flour					
6%	5.5 ± 0.08 ^{de}	67.6 ± 0.8 ^{b}	16.8 ± 0.6 ^{cd}	9.0 ± 1.3 ^{a}	1.7 ± 0.03 ^{d}

Means with different superscripts within each column are significantly different ($p<0.05$).

[1]Values are means of three replicates ± standard deviation

[2]Protein was calculated as total nitrogen x 6.25

The protein content of the raw burgers ranged from 17.2 to 17.8% for those containing lentil flour, 16.6 to 16.8% for those with wheat-based binders, and the no-binder control contained 17.5% protein. Increasing the binder level from 6 to 12% tended to increase protein content, but the change was only significant for the burgers with micronized green lentil. The higher protein content of lentil seed compared to that of the same mass of meat or the same mass of wheat-derived binder explains the elevated protein levels observed when lentil was added to beef burgers. Lastly, burgers containing 6% toasted wheat crumb binder displayed the lowest protein content (16.6%). In the same manner, because the wheat-based binders had lower protein contents than beef or lentil seed, burgers containing wheat binders would be expected to have an overall lower protein content (16.6 – 16.8%) than when lentil binders were used (17.2 – 17.9%).

Ash levels were lowest in burgers containing 0% or 6% binder addition (1.7 – 1.8%). When 12% binder was added to the burgers, ash values increased significantly ($p<0.05$) to 1.9%, with the exception of the burgers containing non-micronized large green lentil. The higher ash content displayed in burgers containing binders was due to the ash contributed by the plant-based binders.

The pH of burgers ranged from 5.5 to 5.7. Increasing the levels of binder increased the pH, but these changes were significant only for burgers containing red lentil. This indicates that red lentil exerted a more alkaline effect than did green lentils. Micronization treatment of lentil had no effect on the pH or proximate composition of raw burgers.

4.2.1.2 Cooked burgers

In comparison with raw burgers, cooking of burgers cooked to 71 °C in an impingement oven exhibited increases in pH, protein, fat, and ash, at the expense of reduced moisture (Table 4.8). All cooked burgers ranged from pH 5.9 to 6.0, 57.0 to 60.5% moisture, 20.7 to 27% protein, 9.4 to 12.1% fat, and 2.0 to 2.2% ash. When the binder level was increased from 6 to 12% in formulations, the moisture content decreased from ~60% to a range of 57 – 59%, and protein content decreased from 23 to 21%.

Table 4.8: Proximate composition[1,2] and pH of cooked low-fat beef burgers formulated with various lentil and wheat binders at levels of 6 or 12%.

Binder Level	pH	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Control					
0%	5.9 ± 0.1 ^{d}	58.1 ± 0.6 ^{d}	27.0 ± 2.2 ^{a}	12.1 ± 2.7 ^{a}	2.1 ± 0.1 ^{ab}
Green Lentil					
Non-micronized					
6%	5.9 ± 0.1 ^{bcd}	59.9 ± 0.9 ^{ab}	22.4 ± 1.1 ^{bcd}	9.7 ± 1.6 ^{b}	2.1 ± 0.1 ^{ab}
12%	6.0 ± 0.1 ^{a}	58.8 ± 1.0 ^{cd}	20.7 ± 0.6 ^{f}	9.8 ± 2.0 ^{b}	2.1 ± 0.1 ^{ab}
Micronized					
6%	5.9 ± 0.0 ^{bcd}	59.6 ± 0.5 ^{b}	22.5 ± 0.6 ^{bc}	10.2 ± 2.1 ^{b}	2.2 ± 0.1 ^{a}
12%	6.0 ± 0.1 ^{ab}	58.1 ± 0.1 ^{d}	21.4 ± 1.2 ^{def}	9.4 ± 1.4 ^{b}	2.2 ± 0.1 ^{ab}
Red Lentil					
Non-micronized					
6%	6.0 ± 0.1 ^{bcd}	60.5 ± 1.0 ^{a}	23.1 ± 1.1 ^{b}	9.6 ± 1.6 ^{b}	2.1 ± 0.1 ^{ab}
12%	6.0 ± 0.1 ^{abc}	58.5 ± 1.1 ^{d}	21.1 ± 0.8 ^{ef}	9.5 ± 1.8 ^{b}	2.2 ± 0.1 ^{ab}
Micronized					
6%	5.9 ± 0.1 ^{bcd}	59.5 ± 0.9 ^{bc}	22.7 ± 1.2 ^{bc}	10.2 ± 1.9 ^{b}	2.2 ± 0.1 ^{ab}
12%	6.0 ± 0.1 ^{abc}	57.0 ± 0.7 ^{e}	21.3 ± 1.2 ^{ef}	10.0 ± 1.4 ^{b}	2.2 ± 0.0 ^{a}
Toasted Wheat Crumb					
6%	5.9 ± 0.0 ^{d}	59.8 ± 1.0 ^{ab}	21.9 ± 1.6 ^{cde}	9.6 ± 2.6 ^{b}	2.1 ± 0.1 ^{b}
Wheat Flour					
6%	5.9 ± 0.1 ^{cd}	60.3 ± 1.1 ^{ab}	21.2 ± 0.9 ^{ef}	10.1 ± 1.6 ^{b}	2.0 ± 0.0 ^{b}

Means with different superscript within each column are significantly different ($p<0.05$).
[1]Values are means of three replicates ± standard deviation
[2]Protein was calculated as total nitrogen x 6.25

When cooked, the control burgers were similar in moisture content (58.1%) to most burgers with 12% lentil, and exhibited the highest fat (12.1%) and protein (27%) contents compared with all other cooked treatments (9.4 – 10.2% fat and 20.0 – 22.5% protein) (Table 4.8). The level of lentil binder used-had no significant effect on the pH, fat, or ash contents of the cooked burgers. Generally, there were no differences in the composition of cooked burgers containing either red or green lentil flour. However, the samples with non-micronized or micronized lentil flours differed significantly in moisture content ($p<0.001$).

4.2.2 Cooking properties

4.2.2.1 Cooking yield

Although binder type generally had no effect on the proximate composition of burgers in the raw or cooked state, the proximate composition was significantly different when comparing raw and cooked burgers. The extent of the proximate gains and losses as a result of cooking the raw beef burgers varied depending on binder type and level used. Cooking properties, cooking yield, and dimensional changes, were measured to demonstrate the degree of these changes (Table 4.9).

Cooking yield ranged from 64.5 to 86.4% (Table 4.9). The control burger had the lowest cooking yield (64.5%), while cooking yield was incrementally higher ($p<0.05$) for burgers containing 6% lentil (77.3 – 79.6%) and 12% lentil (85.5 – 86.4%). The cooking yield for 6% lentil burgers were in the same range as for burgers formulated with 6% reference flour (wheat flour or toasted wheat crumb), indicating comparable abilities to retain moisture and fat. Results from the literature show even higher values. Serdaroglu et al. (2005) observed a 93.2 % cooking yield for meatballs with 10% lentil, while Modi et al. (2003) observed a 92% cooking yield for buffalo beef burgers (10 – 12% fat) with 8% Bengal, or green or black gram flour. However, the burgers in the latter study were produced with buffalo meat that was pre-steamed and subsequently rolled in bread crumbs and deep-fried. Moreover, meatballs are spherical and have a larger thickness compared with the flat, round disc-shape of meat burgers. Therefore, free water or fat in the meat matrix could take a longer time to reach the meatball surface, which would result in relatively lower drip losses in meatballs than in burgers.

Table 4.9: Effect of binders on cooking yield, diameter, and thickness values[1,2] of low-fat beef burgers.

Binder	Cook Yield (%)	Shrinkage in Diameter (%)	Shrinkage in Thickness (%)
Control			
0%	64.5 ± 3.7 [d]	15.9 ± 1.1 [a]	16.8 ± 10.3 [a]
Green Lentil			
Non-micronized			
6%	79.5 ± 1.7 [bc]	13.2 ± 0.5 [b]	1.5 ± 2.1 [bc]
12%	86.0 ± 1.9 [a]	11.4 ± 1.5 [c]	0.0 ± 1.4 [bc]
Micronized			
6%	78.4 ± 1.5 [bc]	12.3 ± 1.1 [bc]	3.1 ± 1.4 [bc]
12%	86.4 ± 1.5 [a]	11.4 ± 0.9 [c]	2.8 ± 2.5 [bc]
Red Lentil			
Non-micronized			
6%	79.6 ± 0.6 [bc]	11.4 ± 1.0 [c]	3.6 ± 1.6 [bc]
12%	85.5 ± 1.2 [a]	11.3 ± 1.0 [c]	-1.9 ± 2.6 [c]
Micronized			
6%	77.3 ± 1.0 [c]	13.1 ± 0.4 [b]	0.0 ± 3.6 [bc]
12%	86.2 ± 1.1 [a]	11.5 ± 1.1 [c]	1.8 ± 4.8 [bc]
Toasted Wheat Crumb			
6%	80.2 ± 1.2 [b]	13.2 ± 0.5 [b]	4.4 ± 2.1 [bc]
Wheat Flour			
6%	80.0 ± 0.6 [b]	12.5 ± 0.7 [bc]	6.9 ± 10.7 [b]

[1]Means with different superscripts within each column are significantly different ($p<0.05$).
[2]Values are means of three replicates ± standard deviation (three determinations per replicate)

Diameter shrinkage ranged from 11.4 to 15.9% and shrinkage in thickness from 0 to 16.8%. Burgers containing 12% lentil flour displayed the least shrinkage in diameter upon cooking, whereas the no-binder control showed the greatest shrinkage in both diameter (15.9%) and thickness (16.8%).

The control burger initially contained the greatest amount of water (Table 4.7), but was unable to retain it in the absence of a binder which led to both greater drip loss and dimensional shrinkage than for burgers containing a binder. These results can be attributed to the water holding capacity of lentil flour alone (non-micronized and micronized), which was determined to be approximately 0.7 to 1.0 g/g. The increase in cooking yield associated with displacing meat with lentil flour indicates that lentil flour addition to meat results in a greater ability to minimize drip loss upon heating. Therefore, increasing the quantity of lentil flour in the burger formula will result in a greater capacity to hold water or fat. Similar cooking yield increases were observed in studies involving meatballs or burgers (~10% fat) with the addition of increasing levels of corn flour, wheat and pea fibre, hazelnut pellicle, or whey protein (Besbes et al., 2008; El-Magoli et al., 1996; Mansour & Khalil, 1997; Serdaroglu & Degirmencioglu, 2004; Turhan et al., 2005).

Despite the higher WHC of micronized lentil compared to non-micronized lentil (Table 4.4), the use of micronized lentil did not significantly influence the cooking yields of the burgers when compared to those containing non-micronized lentil. This could be due to the physical structure of comminuted meat systems and their higher susceptibility to losses during cooking (Anderson & Berry, 2001) compared with other meat systems (e.g. emulsified meat products). These higher losses from this product type appear to override the advantages of the higher WHC of micronized lentil flour. Diameter shrinkage ranged from 11.4 to 15.9%, which exceeds that of Modi et al. (2003) (5.5 – 6.5%). The buffalo burger in the latter study was rolled in bread crumb and fried in 500 mL palm oil, whereas the current study used an impingement oven without oil. Lentil type did not have significant effects on the cooking properties of burgers.

4.2.2.2 Moisture and fat retention

The changes in proximate composition upon cooking can be attributed to drip losses during heating, which is primarily comprised of moisture or fat. The moisture and fat retention values were calculated from proximate analysis data and ranged from 52.0 to 78.8% and 82.6 to 94.9%, respectively (Table 4.10).

The no-binder control burger displayed the lowest moisture retention (52.0%). Inclusion of binders in burger formulations at levels of 6% and 12% increased average moisture retention values to approximately 69% and 78%, respectively. Burgers with 6% lentil flour generally had comparable water retention levels to those containing wheat binders, which are used commercially. There were no major differences in burger water retention among lentil types or between micronized and non-micronized lentil.

Although moisture retention increased with binder level, there were no differences in fat retention (82.6 – 94.9%) among treatments ($p<0.05$). Because micronization did not affect burger cooking yield and gravimetric oil absorption capacity (OAC) analysis of lentil flours, fat retention in burgers was not expected to be affected by micronization. These consistently high fat retention values among treatments indicate that fat loss in low-fat burgers is minimal regardless of binder addition.

Serdaroglu et al. (2005) found moisture and fat retention values to be 56.4% and 95.5%, respectively, in low-fat (9% fat) beef burgers containing 10% lentil flour, whereas El-Magoli et al. (1996) found 44.6% moisture retention and 64.6% fat retention in 11% fat beef patties containing 4% whey protein concentrate. The lower moisture retention values in their studies could be due to their hydrating protocol performed on the binders prior to mixing into the meat. The lack of pre-hydration in the current study would allow the dry binder to hydrate within the meat matrix, leading to greater moisture retention upon cooking.

Table 4.10: Moisture and fat retention values[1,2] (%) for cooked beef burgers containing non-micronized and micronized lentil flours.

Binder Level	Moisture Retention (%)	Fat Retention (%)
Control		
0%	52.0 ± 3.5 [d]	89.0 ± 10.3 [ab]
Green Lentil		
Non-micronized		
6%	70.4 ± 1.6 [bc]	86.9 ± 6.3 [ab]
12%	78.8 ± 2.3 [a]	92.9 ± 4.7 [ab]
Micronized		
6%	69.2 ± 1.1 [bc]	91.1 ± 2.9 [ab]
12%	78.2 ± 2.1 [a]	94.9 ± 3.5 [a]
Red Lentil		
Non-micronized		
6%	70.6 ± 0.7 [b]	91.2 ± 9.2 [ab]
12%	78.4 ± 1.6 [a]	91.3 ± 8.7 [ab]
Micronized		
6%	68.0 ± 1.5 [c]	82.6 ± 4.0 [b]
12%	77.5 ± 1.7 [a]	92.7 ± 5.0 [ab]
Toasted Wheat Crumb		
6%	70.2 ± 1.2 [bc]	89.9 ± 7.7 [ab]
Wheat Flour		
6%	71.4 ± 1.1 [b]	90.5 ± 2.4 [ab]

[1]Means with different superscripts within each column are significantly different ($p<0.05$).
[2]Values are means of three replicates ± standard deviation

4.2.3 Colour analysis

4.2.3.1 Raw burgers

Fresh, raw (never frozen) beef burgers were analyzed for colour using the HunterLab colorimeter on day 0, 1, 3, 5, and 7 for each treatment. Three production replicates were conducted. A split plot analysis of variance was conducted on the colour of raw burgers. The independent variables included a between subject variable, burger treatment with 11 levels, with three production replicates, and a within subject variable, storage time, with five levels (0, 1, 3, 4, and 7 days). Treatment by replicate was used as the error term for testing the main effects of treatment and replicate.

Burger treatments, production replicates, and storage days had significant effects ($p<0.05$) on the colour (L*, a*, b*) of raw burgers. In addition, the treatment by day interaction, and the treatment by replicate interaction were significant, indicating that colour results should be interpreted on a treatment by storage-day basis. For this reason, colour differences (L*, a*, b*) by burger treatment and day of storage are discussed.

The lightness (L*) of burgers with non-micronized green and red lentil (6%) was comparable to the control on days 1, 3, 5, and 7, while those with micronized green and red lentil (6 and 12%) displayed greater lightness than the control on the same days (Table 4.11). Addition of micronized red lentil flour to burgers increased lightness more than if non-micronized lentil flour was used, whereas this was not the case with green lentils. Specifically, in comparison to the use of non-micronized lentil, micronized red lentil addition (12%) produced lighter burgers on days 0 and 3, and micronized red lentil addition (6 and 12%) produced lighter burgers on day 1.

Table 4.11: Colour (L*, lightness)[1,2] of raw low-fat beef burgers containing various binders stored over 7 days at 4 °C.

Binder Level	Raw Burger Lightness (L*)				
	Day 0	**Day 1**	**Day 3**	**Day 5**	**Day 7**
Control					
0%	47.5 ± 2.3 ^{d}	44.7 ± 2.7 ^{e}	44.5 ± 4.1 ^{d}	44.4 ± 5.2 ^{c}	44.9 ± 4.9 ^{d}
Green Lentil					
Non-micronized					
6%	48.6 ± 1.0 ^{cd}	47.2 ± 1.5 ^{cde}	46.1 ± 2.5 ^{cd}	46.6 ± 3.0 ^{bc}	47.2 ± 2.8 ^{cd}
12%	50.0 ± 0.4 ^{bcd}	47.8 ± 0.7 ^{cd}	47.4 ± 1.2 ^{bc}	47.4 ± 1.5 ^{bc}	48.0 ± 1.7 ^{bc}
Micronized					
6%	49.8 ± 2.0 ^{bcd}	48.4 ± 3.0 ^{bcd}	47.7 ± 3.9 ^{bc}	47.3 ± 3.8 ^{bc}	47.2 ± 4.0 ^{cd}
12%	50.7 ± 1.1 ^{abc}	48.9 ± 0.5 ^{bc}	48.6 ± 0.9 ^{abc}	48.2 ± 1.3 ^{ab}	47.6 ± 1.3 ^{c}
Red Lentil					
Non-micronized					
6%	48.4 ± 1.5 ^{cd}	46.2 ± 2.2 ^{de}	46.0 ± 3.3 ^{cd}	46.3 ± 3.6 ^{bc}	46.4 ± 3.3 ^{cd}
12%	50.2 ± 2.3 ^{bc}	48.1 ± 2.8 ^{bcd}	48.3 ± 3.4 ^{bc}	48.3 ± 3.7 ^{ab}	48.3 ± 3.6 ^{bc}
Micronized					
6%	50.2 ± 0.5 ^{bc}	49.2 ± 0.8 ^{bc}	48.5 ± 1.3 ^{abc}	48.2 ± 1.1 ^{ab}	48.0 ± 1.8 ^{bc}
12%	53.1 ± 0.9 ^{a}	51.9 ± 0.8 ^{a}	51.3 ± 1.1 ^{a}	50.9 ± 1.1 ^{a}	50.3 ± 1.5 ^{ab}
Toasted Wheat Crumb					
6%	50.1 ± 3.5 ^{bc}	48.6 ± 3.8 ^{bcd}	48.5 ± 4.2 ^{abc}	48.2 ± 4.8 ^{ab}	48.7 ± 4.3 ^{abc}
Wheat Flour					
6%	51.9 ± 1.5 ^{ab}	50.7 ± 1.3 ^{ab}	50.2 ± 2.6 ^{ab}	51.0 ± 2.4 ^{a}	50.9 ± 2.7 ^{a}

[1]Means within the same column with the same superscripts are not significantly different ($P < 0.05$).
[2]Values are means of three replicates.

The yellowness (b*) of burgers containing non-micronized green or red lentil flour (6%) was comparable to the control and wheat-based reference burgers at days 1 to 7 (Table 4.12). Moreover, the yellowness was greater in burgers with red lentil at days 1 to 7 compared to all other treatments. When micronized lentil was added (6 and 12%), burgers displayed greater yellowness at days 1, 3, and 5 comparedwith the addition of non-micronized lentil, with the exception of green lentil (6%) addition.

Overall, there were incremental losses in redness (a*) for all the raw burger treatments as they were stored over 7 days (Table 4.13, Figure 4.7a, b, c). At day 0, a* values were the highest (25 – 29) and decreased to 10 to 17 units by day 7. At days 3, 5, and 7, redness of all burgers with micronized lentil was significantly higher than that of the burger control or with non-micronized lentil addition ($p<0.05$), with the exception of those with 6% green lentil flour. At day 7, burgers with micronized lentils at 6 or 12% addition levels remained significantly more red than the control or reference burgers. In general, the burgers with toasted wheat crumb or regular wheat flour followed a similar pattern as the burgers with non-micronized lentil, at days 3, 5, and 7, displaying lower redness than their micronized counterparts. Therefore, the presence of any non-micronized lentil binder promoted deterioration of the meat colour over time, and micronization of the lentil to prolonedg redness beyond the rate exhibited by the control.

Table 4.12: Colour (b*, yellowness)[1,2] of raw low-fat beef burgers containing various binders stored from 0 to 7 days at 4 °C.

Binder Level	Raw Burger (b*)				
	Day 0	**Day 1**	**Day 3**	**Day 5**	**Day 7**
Control					
0%	24.7 ± 4.3 ^{ef}	22.0 ± 3.3 ^{fgh}	19.5 ± 2.5 ^{f}	18.1 ± 1.7 ^{h}	17.5 ± 0.7 ^{f}
Green Lentil					
Non-micronized					
6%	25.4 ± 2.5 ^{de}	22.8 ± 2.1 ^{def}	20.5 ± 1.0 ^{ef}	18.7 ± 1.1 ^{fgh}	18.5 ± 1.3 ^{ef}
12%	27.1 ± 3.0 ^{bc}	24.1 ± 1.6 ^{cd}	21.3 ± 0.3 ^{de}	20.0 ± 0.4 ^{def}	19.6 ± 0.5 ^{cde}
Micronized					
6%	25.3 ± 2.8 ^{de}	23.6 ± 1.8 ^{cde}	22.6 ± 1.0 ^{cd}	21.1 ± 1.0 ^{cde}	19.5 ± 0.2 ^{cde}
12%	26.6 ± 2.7 ^{bcd}	25.5 ± 1.5 ^{b}	24.7 ± 0.9 ^{b}	23.9 ± 1.3 ^{b}	21.5 ± 1.8 ^{ab}
Red Lentil					
Non-micronized					
6%	25.8 ± 2.0 ^{cde}	22.5 ± 1.2 ^{efg}	20.2 ± 0.5 ^{ef}	19.8 ± 0.8 ^{efg}	19.9 ± 1.1 ^{bcde}
12%	27.8 ± 2.5 ^{ab}	23.9 ± 1.6 ^{cd}	21.6 ± 0.7 ^{de}	21.2 ± 1.0 ^{cd}	20.9 ± 0.7 ^{bc}
Micronized					
6%	26.5 ± 2.5 ^{bcd}	24.9 ± 0.7 ^{bc}	23.6 ± 0.7 ^{bc}	22.2 ± 0.6 ^{c}	20.4 ± 0.1 ^{bcd}
12%	28.9 ± 1.8 ^{a}	27.4 ± 1.8 ^{a}	26.7 ± 1.3 ^{a}	25.4 ± 1.7 ^{a}	23.1 ± 1.8 ^{a}
Toasted Wheat Crumb					
6%	23.2 ± 2.8 ^{g}	21.3 ± 2.5 ^{gh}	19.4 ± 1.1 ^{f}	18.5 ± 0.8 ^{gh}	18.3 ± 1.4 ^{ef}
Wheat Flour					
6%	23.3 ± 2.7 ^{fg}	21.0 ± 1.8 ^{h}	19.4 ± 0.8 ^{f}	18.6 ± 0.7 ^{gh}	19.0 ± 0.6 ^{def}

[1]Means within the same column with the same superscripts are not significantly different ($P< 0.05$).
[2]Values are means of three replicates.

Table 4.13: Colour (a*, redness)[1,2] of raw low-fat beef burgers containing various binders stored from 0 to 7 days at 4 °C.

Binder Level	Raw Burger Redness (a*)				
	Day 0	**Day 1**	**Day 3**	**Day 5**	**Day 7**
Control					
0%	29.4 ± 6.0 ^{a}	26.0 ± 5.0 ^{ab}	20.9 ± 4.3 ^{b}	15.4 ± 5.2 ^{b}	10.4 ± 1.4 ^{d}
Green Lentil					
Non-micronized					
6%	27.9 ± 4.4 ^{abc}	24.4 ± 3.4 ^{bc}	19.0 ± 2.6 ^{bc}	13.4 ± 1.5 ^{bc}	10.5 ± 0.3 ^{d}
12%	27.6 ± 4.2 ^{abc}	23.6 ± 2.6 ^{cd}	17.4 ± 1.0 ^{cd}	13.2 ± 0.9 ^{bc}	11.0 ± 0.4 ^{d}
Micronized					
6%	27.3 ± 5.5 ^{bcd}	25.6 ± 4.0 ^{ab}	23.7 ± 2.7 ^{a}	20.3 ± 3.9 ^{a}	14.5 ± 3.5 ^{bc}
12%	26.5 ± 4.7 ^{cde}	25.7 ± 3.0 ^{ab}	24.3 ± 1.9 ^{a}	22.8 ± 2.2 ^{a}	17.4 ± 4.6 ^{a}
Red Lentil					
Non-micronized					
6%	27.4 ± 4.7 ^{bc}	22.2 ± 4.0 ^{de}	15.9 ± 2.0 ^{d}	12.4 ± 1.0 ^{bc}	11.4 ± 0.1 ^{d}
12%	27.1 ± 4.3 ^{bcd}	21.3 ± 2.8 ^{e}	15.8 ± 0.9 ^{d}	13.5 ± 0.6 ^{bc}	12.4 ± 0.5 ^{cd}
Micronized					
6%	27.6 ± 4.6 ^{abc}	26.0 ± 2.4 ^{ab}	24.0 ± 1.4 ^{a}	20.6 ± 2.4 ^{a}	14.4 ± 1.9 ^{bc}
12%	28.6 ± 3.2 ^{ab}	27.1 ± 2.5 ^{a}	25.8 ± 1.6 ^{a}	23.1 ± 2.5 ^{a}	16.4 ± 2.7 ^{ab}
Toasted Wheat Crumb					
6%	25.5 ± 5.4 ^{de}	22.8 ± 4.4 ^{cde}	18.1 ± 4.0 ^{bcd}	13.5 ± 3.9 ^{bc}	10.2 ± 1.1 ^{d}
Wheat Flour					
6%	25.2 ± 5.0 ^{e}	21.7 ± 3.9 ^{e}	16.6 ± 2.8 ^{cd}	11.2 ± 1.3 ^{c}	9.9 ± 0.5 ^{d}

[1]Means within the same column with the same superscripts are not significantly different ($P < 0.05$).
[2]Values are means of three replicates.

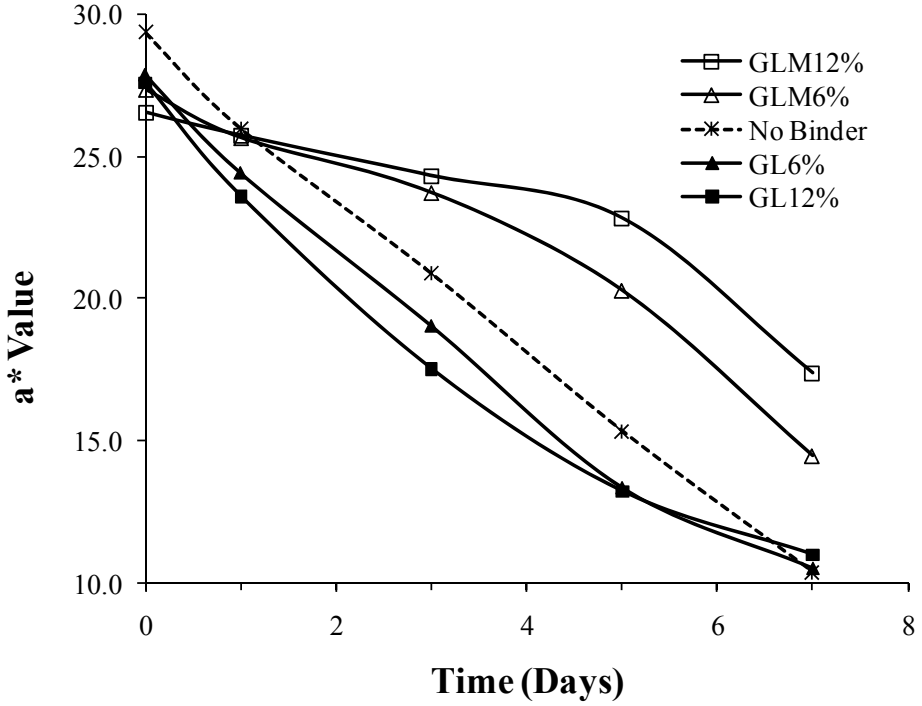

Figure 4.7a: HunterLab a* values for fresh burgers containing green lentil flour and stored at 4 °C from 0 to 7 days (GLM = Green lentil micronized; GL = green lentil non-micronized).

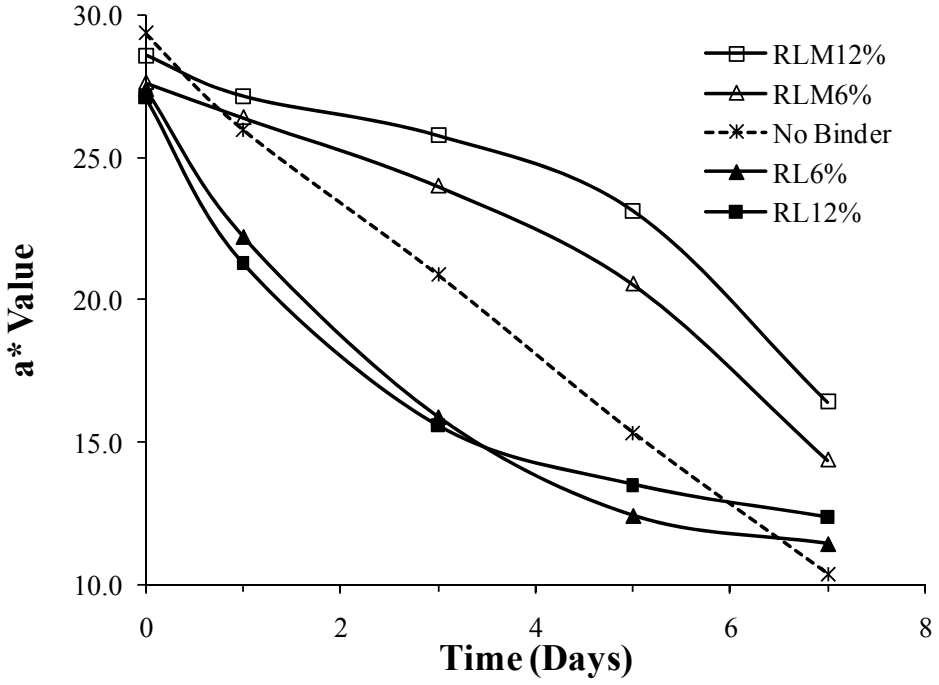

Figure 4.7b: HunterLab a* values for fresh burgers containing red lentil flour and stored at 4 °C over 7 days (RLM = red lentil micronized; RL = red lentil non-micronized)

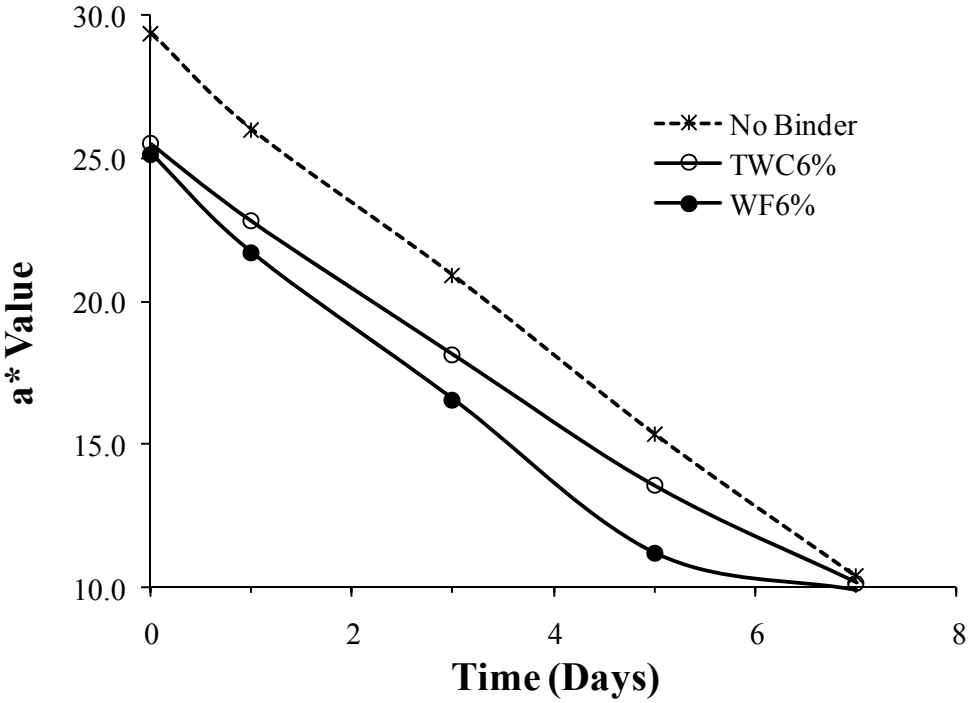

Figure 4.7c: HunterLab a* values for fresh burgers containing TWC and WF and stored at 4 °C over 7 days (TWC = toasted wheat crumb; WF = wheat flour).

Maintenance of redness in raw burgers is important because it is an indicator of meat oxidative processes which eventually lower the shelf life. This is particularly important for the fresh meat market, where visual quality is highly valued. The maintenance of redness in raw burgers observed during day 0 to 5 in the presence of micronized lentil observed in this study is particularly advantageous since the loss of redness has been found to be greatest within the first 5 or 6 days of refrigerated storage (Fernández-Lopez et al., 2006). Their study measured the colour of raw burgers (beef mixed with ostrich meat, 7% fat) stored at 4° C over 9 days and found that the greatest decrease in redness occurred at days 1 to 6 and levelled off thereafter. Similarly, Rhee et al. (1985) found instrumental redness of ground beef (25% fat) to decrease the greatest (49%) in the first three days. When they added 3% cottonseed flour to the ground beef, the rate of red discolouration was decreased by 43% during the first three days and this elevated redness was maintained up to day 6.

Micronization applies heat to the lentil seed surface and results not only in a physical colour change in the flour, but also changes in enzyme activity. Micronization was shown to significantly reduce lipoxygenase activity in the lentil seed. This level of inactivation may inhibit lipid hydrolysis and increase the stability of the flour in food applications. Lipoxygenase can oxidize linoleic acid, thereby forming fatty acid radical byproducts which can then propagate oxidative reactions (Damodaran et al., 2008). These oxidative byproducts, such as aldehydes, can then react with histidine of myoglobin, the main meat pigment in meat. Oxidation of myoglobin results in metmyoglobin which is associated with meat discolouration. Furthermore, exposure of the heme iron upon myoglobin decomposition will allow interaction with other hydroperoxides, leading to further loss of redness.

The prolonged redness observed in burgers containing micronized lentil flours, beyond that of the control, could also be due to the antioxidants present. The development of Maillard reaction byproducts as a result of micronization could suppress lipid oxidation. Acar et al. (2009) demonstrated that dry roasting of pulses for up to 60 min increased their antioxidant activity (~20%) and attributed this to the development of Maillard reaction products, such as melanoidans, possessing metal-chelating properties.

Therefore, because lipoxygenase activity has been reduced or eliminated in lentil via micronization, and there was potential development of Maillard reaction products, oxidation was delayed in the low-fat burgers leading to improved colour stability. This can have positive implications for the use of lentil flour in the development of meat products in the fresh meat market.

4.2.3.2 Cooked burgers

Cooking changes the colour of beef burgers and can be influenced by the ingredients added to the formulation. Colour analysis results for cooked burgers containing various binders are found in Table 4.14. Colour measurements were taken from the exterior and interior of cooked burgers (two samples) from three production replicates.

Table 4.14: Effect of flour binders on colour[1,2] (exterior and interior) of cooked low-fat beef burgers.

Binder	Colour - Exterior			Colour - Interior		
	L*	a*	b*	L*	a*	b*
Control						
0%	45.5 ± 2.8 ^{bcd}	10.2 ± 0.1 ^{ef}	13.8 ± 0.8 ^{f}	53.8 ± 2.1 ^{a}	9.3 ± 0.4 ^{e}	14.0 ± 0.7 ^{e}
Green Lentil						
Non-micronized						
6%	46.4 ± 2.2 ^{abcd}	10.5 ± 0.3 ^{de}	14.7 ± 0.7 ^{def}	53.5 ± 1.8 ^{abc}	10.2 ± 0.1 ^{c}	14.8 ± 0.5 ^{de}
12%	46.1 ± 1.8 ^{abcd}	10.8 ± 0.1 ^{cd}	17.9 ± 0.8 ^{ab}	52.9 ± 2.1 ^{abc}	10.3 ± 0.7 ^{c}	15.3 ± 0.7 ^{cd}
Micronized						
6%	47.3 ± 4.3 ^{a}	9.9 ± 0.6 ^{f}	14.2 ± 0.5 ^{ef}	53.7 ± 2.8 ^{a}	9.2 ± 0.5 ^{e}	14.9 ± 0.6 ^{de}
12%	45.0 ± 2.9 ^{bcde}	10.4 ± 0.4 ^{def}	17.1 ± 1.2 ^{ab}	51.3 ± 1.4 ^{d}	9.3 ± 0.1 ^{de}	16.0 ± 0.6 ^{bc}
Red Lentil						
Non-micronized						
6%	44.5 ± 1.9 ^{de}	11.4 ± 0.1 ^{b}	16.7 ± 0.9 ^{bc}	52.8 ± 1.8 ^{abc}	11.7 ± 0.5 ^{b}	15.6 ± 0.2 ^{cd}
12%	44.6 ± 4.1 ^{cde}	12.5 ± 0.3 ^{a}	18.2 ± 0.7 ^{a}	51.7 ± 2.5 ^{cd}	12.5 ± 0.4 ^{a}	16.9 ± 0.2 ^{a}
Micronized						
6%	45.2 ± 2.6 ^{bcde}	10.5 ± 0.2 ^{de}	15.5 ± 0.3 ^{cde}	53.1 ± 2.2 ^{ab}	9.7 ± 0.3 ^{cde}	15.2 ± 0.3 ^{cd}
12%	45.7 ± 2.6 ^{abcd}	11.3 ± 0.6 ^{bc}	17.9 ± 1.0 ^{ab}	51.9 ± 1.6 ^{bcd}	10.2 ± 0.5 ^{c}	16.5 ± 0.5 ^{ab}
Toasted Wheat Crumb						
6%	43.9 ± 3.2 ^{e}	10.8 ± 0.4 ^{d}	15.7 ± 0.4 ^{cd}	52.0 ± 1.8 ^{bcd}	9.7 ± 0.2 ^{cde}	14.7 ± 0.3 ^{de}
Wheat Flour						
6%	46.2 ± 3.6 ^{abc}	10.6 ± 0.4 ^{de}	15.5 ± 1.3 ^{cde}	52.5 ± 2.1 ^{abcd}	10.0 ± 0.1 ^{cd}	14.2 ± 0.3 ^{e}

[1]Means with different superscript within each column are significantly different ($p<0.05$).
[2]Values are means of three replicates ± standard deviation (two burgers analyzed per replicate)

4.2.3.2.1 Exterior colour

The exterior of the cooked burgers displayed similar lightness (L*) among all treatments, with the exception of burgers with micronized green lentil at 6%, which were lighter than the control ($p<0.05$). Exterior redness was greater in burgers with red lentil flour compared with the control or those with green lentil (with the exception of the burger containing micronized red lentil flour at 6% which had similar redness to the control). The control burger, along with all burgers with 6% green lentil, displayed similar b* values (13.8 – 14.7). However, only the control had significantly lower b* than all other treatments (14.2 – 18.2). Moreover, increasing the lentil level from 6 to 12% also increased yellowness significantly for burgers containing red or green lentil flour. Overall, binder colour significantly influenced burger exterior redness (a*) and yellowness (b*) of burgers as indicated by significant orthogonal contrasts ($p<0.001$ or $p<0.01$, respectively).

4.2.3.2.2 Interior colour

The colour of the interior of cooked burgers was analyzed after cutting the burgers horizontally. The lightness (L*) of the control burger was similar to burger treatments containing 6% of any binder, with the exception of burgers with toasted wheat crumb, which were significantly darker than the control. Burgers with any red lentil at 12%, or 12% micronized green lentil, were darker than the control. Redness (a*) was lower in burgers with flour from micronized red and green lentil (9.2 – 10.2) than in respective samples with non-micronized flours (10.2 – 12.5). Heat treatment had a significant effect on cooked burger redness as was shown by results from contrast statistical analysis of burger treatments between micronized and non-micronized samples ($p<0.001$, data not shown). According to orthogonal contrast analysis, burgers with green or red lentil binder also showed significant effects on burger colour ($p<0.001$). Increasing the level of non-micronized red lentil in burgers from 0 to 12% increased the internal redness (a*) of the cooked burgers, however this was not seen with green lentil flour.

Visually and instrumentally, micronizing red lentil changed the seed and flour colour from a vibrant red to a pale orange hue (Table 4.3, Figure 4.1). This colour change due to micronization of lentil was reflected in the meat product (cooked) that it was incorporated into, as was evidenced by a lower a* value in the burger exterior (with exception of 12% green lentil addition) and in the burger interior compared with burgers with non-micronized lentils.

Similar to the present study, addition of cowpea flour (0 – 20%) to chicken nuggets increased darkness and redness, and decreased yellowness, in the cooked product (Prinyawiwatkul et al., 1997). Due to the perceived association of redness with blood in raw meat (Kubberød et al. 2002), a less red colour would be advantageous in a cooked meat product. Overall, the use of green lentil or micronized red lentil flour in a burger binder would be suitable since the colour results are comparable to those of the no binder control or toasted wheat crumb reference.

4.2.4 Analysis of thiobarbituric acid reactive substances in raw beef burgers

The TBARS test was used as a chemical indicator for the degree of lipid oxidation in raw, frozen beef burgers. Analyses were done on each of the three replicates of burger production. Samples were stored for 9 to 11 weeks at -30 °C prior to testing. Results are presented in Table 4.15.

Overall, burgers containing 6 and 12% non-micronized lentil flour displayed the highest TBARS values. There were no differences in TBARS values among raw burgers containing either 6 or 12% lentil flour. However, addition of red lentil flour significantly increased TBARS values (1.7 – 1.9 mg/kg) compared with the control ($p<0.05$).

Micronization of green and red lentil significantly ($p<0.05$) reduced the formation of TBARS in raw, frozen beef burgers (0.5 – 0.8 mg/kg) compared with non-micronized treatments (1.5 – 1.9 mg/kg, Table 4.15). Burgers with wheat-based binders exhibited TBARS (0.9 – 1.0 mg/kg) that were comparable to those with micronized lentil or the no-binder control.

Table 4.15: Thiobarbituric acid reacting substances for raw, frozen beef burgers containing various flours stored for 9 – 11 weeks.

Treatment	TBARS values*
Control	
0%	1.2 ± 0.4 [bc]
Green Lentil	
Non-micronized	
6%	1.5 ± 0.6 [ab]
12%	1.5 ± 0.3 [ab]
Micronized	
6%	0.8 ± 0.3 [cd]
12%	0.5 ± 0.4 [d]
Red Lentil	
Non-micronized	
6%	1.7 ± 0.5 [a]
12%	1.9 ± 0.3 [a]
Micronized	
6%	0.6 ± 0.3 [d]
12%	0.8 ± 0.2 [cd]
Toasted Wheat Crumb	
6%	0.9 ± 0.6 [cd]
Wheat Flour	
6%	1.0 ± 0.5 [cd]

* (mg malonaldehyde equivalents / kg burger)
Means with different superscripts within each column are significantly different at $p<0.05$.
Values are means of duplicate determinations (of three replications) ± standard deviation.

The effectiveness of micronization is demonstrated here with these results indicating that burgers containing micronized lentil had suppressed lipid oxidation. Higher TBARS values indicate higher detection of unsaturated aldehydes such as malondialdehyde (MDA). MDA is a primary lipid oxidation product involving fatty acids with three or more double bonds and exhibits a strong absorbance at 532 nm (Shahidi & Zhong, 2007). However, values could be overestimated due to the non-specific reaction of TBA with other non lipid-derived unsaturated aldehydes such as ascorbic acid or sugars (De la Heras et al., 2003). On the contrary, values could be underestimated as a result of MDA being further propagated into secondary lipid oxidation products, thus making it unavailable to couple with the TBA reagent during analysis (Damodaran et al., 2008). In this study, TBARS analysis was conducted on raw burgers that were produced 9 to 11 weeks prior and stored in vacuum bags at -20 °C during this period. However, because analysis was conducted only at this point in time, using the TBARS test to measure primary oxidation products may not best reflect the true extent of oxidation over this 9 to 11 week period. Measuring TBARS at several points in time could better reveal the trends for oxidation, thus allowing the effects of various binders to be better understood. Similar effects of heat-treated binders used in meat products were observed in the study by Modi et al. (2003) where roasting of legume flours (green/black/bengal gram) prior to incorporation into buffalo meat burgers (10 – 12% fat) resulted in lower TBARS over 4 months at 4° C storage compared with non-heated binders. Suppression of lipid oxidation could be attributed to the development of Maillard reaction byproducts as a result of micronization (Acar et al., 2009).

4.2.5 Instrumental texture analysis

The texture of cooked beef burgers can vary depending on the combined function of the ingredients used in formulating the burger. During cooking of meat, muscle proteins become fully denatured and aggregate, and the liquid that was bound to these proteins is released (Aberle et al., 2001). Consequently, meat fibres are more compressed, which leads to the exclusion of free water from the meat matrix, and the firming of meat texture (Aberle et al., 2001). The presence of binders has been shown to minimize water exclusion through increased cooking yield and water retention measurements, as presented in earlier sections. These effects of binder addition can also be demonstrated through instrumental textural analysis of cooked burgers.

4.2.5.1 Shear force

Table 4.16 shows the effect of binders on the shear force of low-fat beef burgers. The control burger (no binder) showed the highest shear force (54.0 N) compared with all other treatments (28.9 – 38.1 N) ($p<0.05$). Treatments with any binder did not significantly differ from each other. Burgers with binders also had higher moisture retention and cooking yields (Table 4.9 and 4.10), and therefore the retained moisture can be accommodated within the muscle fibres in the meat, thus explaining the lower force required in shearing these burgers. Similar trends were observed by Mansour and Khalil (1997) where increasing additions of hydrated wheat fibre (up to 15%) in low-fat beef burgers decreased shear force values, which was ideal for their aim to reduce fat.

Table 4.16: Effect of binders on shear force[1,3] and texture profile analysis[2,3] of cooked beef burgers.

Binder	Shear Force (N)	Texture Profile Analysis		
		Hardness (N)	Cohesiveness	Springiness (%)
Control				
0%	54.0 ± 11.5 [a]	82.0 ± 9.9 [bcd]	0.56 ± 0.0 [a]	75.4 ± 1.5 [a]
Green Lentil				
Non-micronized				
6%	32.2 ± 7.2 [b]	69.8 ± 10.7 [de]	0.38 ± 0.0 [bc]	64.5 ± 2.6 [c]
12%	29.3 ± 2.1 [b]	81.0 ± 5.4 [bcd]	0.35 ± 0.0 [c]	64.5 ± 2.6 [c]
Micronized				
6%	38.1 ± 2.0 [b]	77.4 ± 3.2 [cde]	0.44 ± 0.0 [b]	67.1 ± 3.4 [bc]
12%	32.5 ± 3.7 [b]	107.0 ± 5.6 [a]	0.37 ± 0.0 [bc]	66.2 ± 1.5 [bc]
Red Lentil				
Non-micronized				
6%	31.7 ± 7.5 [b]	72.5 ± 8.9 [de]	0.41 ± 0.0 [b]	65.9 ± 2.0 [bc]
12%	28.9 ± 1.8 [b]	87.8 ± 12.7 [bc]	0.36 ± 0.0 [bc]	64.7 ± 3.3 [bc]
Micronized				
6%	37.4 ± 5.3 [b]	90.9 ± 4.6 [b]	0.44 ± 0.0 [b]	69.1 ± 2.2 [b]
12%	32.5 ± 1.8 [b]	112.2 ± 14.8 [a]	0.38 ± 0.0 [bc]	65.2 ± 4.9 [bc]
Toasted Wheat Crumb				
6%	31.9 ± 0.8 [b]	69.9 ± 11.2 [de]	0.34 ± 0.1 [b]	58.6 ± 4.8 [d]
Wheat Flour				
6%	31.3 ± 8.5 [b]	65.5 ± 13.2 [e]	0.33 ± 0.1 [b]	56.9 ± 5.7 [d]

[1]Means with different superscript within each column are significantly different ($p<0.05$).
[2]Values are means of three replicates ± standard deviation (four determinations for shear force, and eight determinations for texture profile analysis)
[3]N = Newtons

4.2.5.2 Texture profile analysis

The TMS-Pro Texture Press was used to compress standard pieces of burgers two times and the associated forces were measured or calculated to obtain values such as hardness, cohesiveness, and springiness. Results for texture profile analysis of burgers are presented in Table 4.16. The hardness of burgers with green or red lentil ranged from 70 to 107 N and 72 to 112 N, respectively. Burger hardness was significantly increased when the lentil addition level was increased from 6% to 12%, with the exception of burgers containing non-micronized green lentil, where there was no significant difference due to binder level. The burgers with 12% micronized green or red lentil exhibited the greatest hardness (107 – 112 N) among all other treatments. All burgers with 0 or 6% lentil addition (70 – 77 N) exhibited hardness similar to the reference burgers containing the wheat-based binders (66 – 70 N), with the exception of 6% micronized red lentil addition which produced greater hardness (91 N).

Burger hardness was increased only significantly when the micronized binder was added at a level of 12%. This increase in hardness was attributed to the observed tendency for surface-crust formation upon cooking of burgers containing higher amounts of lentil binder. This harder surface would result in higher initial peak force, as it would require greater force to depress the sample surface. Despite this harder surface, panelists noted that burgers containing non-micronized lentil exhibited a 'mushy' character in the burger interior.

Springiness was highest in the control burger (75.4%), lowest in burgers containing toasted wheat crumb (58.6%) and wheat flour (56.9%), with those containing lentil binders falling in between these ranges (64.5 – 69.1%). Burger cohesiveness was greatest for the control burger ($p<0.05$).

4.2.6 Trained sensory panel

4.2.6.1 Initial and overall juiciness

Initial juiciness, or initial release of liquid upon the first bite of a burger, is an important sensory attribute for a burger, as is the overall juiciness perceived throughout mastication when the food is crushed and ground by the teeth (Meilgaard et al., 2007). Table 4.17 displays sensory results related to juiciness and texture based on scores generated from a 13-member trained panel.

Upon adding 6% lentil flour to burgers, initial juiciness increased from 4.8 in the control to 5.2 to 5.3 in burgers containing 6% lentil. With 12% addition, initial juiciness was significantly lower than the control. Moreoever, with the exception of 6% red lentil flour, the use of micronized lentil in burgers significantly decreased initial juiciness compared to the use of non-micronized lentil ($p<0.05$). In comparison, overall juiciness displayed similar trends as initial juiciness ($r = 0.97$, $p<0.001$). Juiciness increased from 4.7 in the control, to 5.1 to 5.4 upon addition of 6% lentil, decreased upon addition of 12% (4.2 – 4.3), and further decreased with the addition of micronized lentil only at the 12% level (3.4 – 3.5). Overall, micronization treatment of lentil showed significant effects on burger juiciness according to contrast analysis ($p<0.001$).

Moisture (Aberle et al., 1989) and fat (Berry, 1992; Cross et al., 1980; Egbert et al., 1991; Serdaroglu, 2005) are known to contribute to juiciness in meat. However, in the case of low-fat burgers, the contribution of fat to juiciness is less likely due to the low level of fat. Increased burger juiciness when the level of binder addition was increased from 0 to 6% corresponded to increased moisture retention and cooking yields in cooked burgers at those levels (Tables 4.9 and 4.10). However, despite increasing moisture retention and cooking yields when the level of binder addition was increased from 6 to 12%, perception of overall burger juiciness was decreased. The reason for the decreased juiciness as lentil addition was increased from 6 to 12% could be due to the lower moisture levels observed in the cooked burgers with 12% binder addition (57 – 59%) compared with 6% addition levels (60 – 61%) (Table 4.8).

Table 4.17: Effect of flour binders on the sensory properties[1,2] of low-fat beef burgers.

Treatment	Initial Juiciness	Overall Juiciness	Initial Hardness	Overall Tenderness	Cohesive - ness
Control					
0%	4.8 ± 0.1 [c]	4.7 ± 0.3 [b]	5.7 ± 0.3 [a]	3.3 ± 0.1 [e]	6.1 ± 0.1 [a]
Green Lentil					
Non-micronized					
6%	5.3 ± 0.3 [ab]	5.4 ± 0.3 [a]	3.1 ± 0.3 [e]	5.9 ± 0.4 [abc]	4.3 ± 0.4 [c]
12%	3.7 ± 0.5 [d]	4.2 ± 0.3 [c]	3.7 ± 0.6 [de]	5.7 ± 0.4 [abc]	4.2 ± 0.2 [c]
Micronized					
6%	4.8 ± 0.3 [c]	5.1 ± 0.2 [ab]	4.0 ± 0.3 [d]	5.4 ± 0.2 [bcd]	4.7 ± 0.2 [bc]
12%	3.0 ± 0.2 [e]	3.4 ± 0.3 [d]	4.9 ± 0.4 [b]	5.0 ± 0.7 [d]	4.7 ± 0.3 [bc]
Red Lentil					
Non-micronized					
6%	5.2 ± 0.1 [ab]	5.5 ± 0.0 [a]	3.5 ± 0.6 [de]	5.4 ± 0.2 [bcd]	4.7 ± 0.2 [bc]
12%	3.8 ± 0.3 [d]	4.3 ± 0.3 [c]	3.7 ± 0.5 [de]	5.6 ± 0.5 [abc]	4.4 ± 0.3 [c]
Micronized					
6%	5.0 ± 0.2 [bc]	5.2 ± 0.2 [a]	4.1 ± 0.1 [cd]	5.4 ± 0.2 [bcd]	5.0 ± 0.4 [b]
12%	3.0 ± 0.3 [e]	3.5 ± 0.2 [d]	4.7 ± 0.6 [bc]	5.2 ± 0.5 [cd]	4.5 ± 0.4 [bc]
Toasted Wheat Crumb					
6%	5.0 ± 0.4 [abc]	5.2 ± 0.2 [a]	3.5 ± 0.8 [de]	5.6 ± 0.4 [abc]	4.7 ± 0.6 [bc]
Wheat Flour					
6%	5.4 ± 0.2 [a]	5.5 ± 0.2 [a]	3.0 ± 0.7 [e]	6.0 ± 0.4 [a]	4.6 ± 0.5 [bc]

[1]Means within the same column with the same superscripts are not significantly different
($P > 0.05$).

[2]Highest possible score = 8 (Extremely juicy, hard, cohesive, tender);
Lowest possible score = 1 (Extremely dry, soft, loose, tough)

The addition of micronized lentil flour decreased burger juiciness despite micronization not having an effect on moisture retention or cooking yield (Table 4.9 and 4.10). Although micronized lentil flour, alone, displayed increasing water holding capacities (Table 4.4), the extra water held by the lentil flour binder especially when micronized, would be comprised of water tightly bound to starch and protein components. For this reason, the bound water may not have been released to impart a perceived juiciness upon mastication.

Other factors affecting juiciness could be structural changes to the meat matrix when binders in general, are added to burgers. During manufacture, added fine lentil flour would be hydrated in the meat mixture and would bind ground meat particles, creating a more unified product. Effectively, pockets in the meat matrix would become occupied with lentil flour where liquid (water, fat) could have been physically trapped if the pockets were vacant. As a result, release of any free liquid will occur during cooking and not during mastication. Consequently, increasing the lentil flour level to 12% in meat burgers would not necessarily produce a juicier burger despite its ability to hold water.

In other studies, juiciness sensory scores were either maintained or decreased upon addition of various binders to pre-formed beef products. Juiciness was maintained upon addition of up to 10% wheat fibre to 8% fat beef burgers when compared with a no-binder control (Mansour & Khalil, 1997). In the study by Shaner and Baldwin (1979), juiciness decreased significantly when 30% chickpea flour was added to a meat loaf formulation. The flour of roasted legumes such as bengal, black, and green gram when added at 8% to buffalo burgers (10 – 12% fat) did not significantly change juiciness when compared with burgers containing non-heated legumes.

4.2.6.2 Initial hardness and overall tenderness

The control burgers had the greatest initial hardness (5.7) or least tender texture (3.3) among all treatments (Table 4.17) ($p<0.05$). Addition of 6% of binder to the burgers significantly decreased initial hardness (3.1 – 4.1), which remained at this level with 12% addition (3.7 – 4.7), with the exception of the addition of 12% micronized

green lentil (4.9). When micronized binders were used, hardness increased significantly ($p<0.05$). Contrast analysis confirmed that binder micronization affects hardness ($p<0.001$). The hardness of the burger with TWC was comparable to that of burgers containing 6 or 12% of non-micronized lentil flour or containing 6% of micronized lentil flour. Conversely, overall burger tenderness increased with 6% binder addition, but neither binder level nor micronization had any effect.

Addition of binder at the 6% level will increase tenderness due to the added retention of water in the burgers. Similarly, it was demonstrated by Desmond and Troy (1998) that adding various non-meat adjuncts (0.5 – 3.0% soy, milk, oat, carrageenan extracts) to low-fat beef burgers (9 – 11% fat) resulted in higher moisture content, cooking yield, and water holding capacity than the control, and produced a more tender product. However, in the current study, when a higher level of lentil binder was used (12%), there was lower moisture overall and more water would be bound to components of the flour, consequently making less free water available and leading to the perception of lower tenderness.

Moreover, when 12% of lentil flour was incorporated into the beef burger, the structure of the meat matrix would be changed. Hydration of lentil flour forms a slurry, thus binding the meat burger components more cohesively. When meat particles and binder and other ingredients are more densely packed, the meat matrix will appear to be tougher, *i.e.* less tender. The use of micronized lentil flour produced an even harder burger, which could be attributed to the greater absorption of water by micronized lentil, as well as the tendency to form a surface crust on cooking.

Beef burgers were cooked in an impingement oven to an internal temperature of 75 ± 1 °C. Due to the varying composition of the burger treatments, the times to reach this critical temperature were different, *i.e.* 10 ± 1 min and 12 ± 0.5 min for burgers containing binder and no binder, respectively. Burgers subjected to longer cooking times to reach the critical cook temperature exhibited lower overall juiciness and greater hardness, as well as lower cook yields. Similar observations were reported by Berry et al. (2001) when they compared the effect of four cooking temperatures on the properties

of beef patties. In the present study, the presence of 6% binder resulted in faster cook times while maintaining superior sensory qualities.

4.2.6.3 Flavour

Addition of new ingredients into meat formulations can influence the flavour of the finished product and was investigated. Sensory results relating to flavour attributes and overall acceptability according to a trained panel are presented in Table 4.18.

Overall flavour intensity scores ranged from 4.6 to 5.3. Off-flavours were significantly greater in burgers containing non-micronized lentil at a level of 6 or 12% (3.2 – 3.9), and the lowest with micronized lentil at 6% (1.6) ($p<0.05$). The burgers with no binder or toasted wheat crumb scored similarlyto micronized lentil, with values of 1.8 and 2.1, respectively. Although increasing micronized lentil use from 6 to 12% significantly increased off-flavour levels to 2.7 to 2.8, it did not exceed the off-flavour values exhibited by the non-micronized binders (3.2 – 3.9) highlighted above. Off-flavour descriptors offered by the panelists such as “beany” pertained to the lentil ingredient.

Flavour desirability was lowest for burgers containing non-micronized lentil at a level of 6 or 12% (4.4 – 5.0) and highest for those with micronized lentil at 6% (5.8 – 6.0) ($p<0.05$), which also corresponded to the highest and lowest off-flavours, respectively. The flavour desirability of burgers made with toasted wheat crumb (5.4) or no binder (5.2) fell within this overall range, although the control had higher flavour desirability than did burgers containing micronized red lentil at a level of 12% addition. Generally, flavour desirability decreased when the lentil incorporation level was increased from 6 to 12%, although this change was only statistically significant with micronized lentil.

Table 4.18: Effect of flour binders on the sensory (flavour) properties[1,2] of low-fat beef burgers.

Treatment	Overall Flavour Intensity	Flavour Desirability	Presence of Off-flavour	Saltiness[3]	Overall Accept - ability
Control					
0%	4.8 ± 0.3 ^{bcd}	5.2 ± 0.3 ^{bc}	1.8 ± 0.3 ^{ef}	2.5 ± 0.1 ^{abc}	4.6 ± 0.0 ^{c}
Green Lentil					
Non-micronized					
6%	5.1 ± 0.3 ^{ab}	5.0 ± 0.8 ^{cd}	3.2 ± 0.7 ^{abc}	2.5 ± 0.1 ^{abc}	5.0 ± 0.8 ^{bc}
12%	4.9 ± 0.2 ^{bcd}	4.7 ± 0.7 ^{cd}	3.7 ± 0.7 ^{a}	2.1 ± 0.0 ^{efg}	4.4 ± 0.6 ^{c}
Micronized					
6%	4.9 ± 0.2 ^{abc}	5.8 ± 0.2 ^{ab}	1.6 ± 0.2 ^{f}	2.6 ± 0.2 ^{ab}	5.9 ± 0.3 ^{a}
12%	4.6 ± 0.2 ^{cd}	5.0 ± 0.0 ^{cd}	2.7 ± 0.7 ^{bcd}	2.3 ± 0.2 ^{cde}	4.8 ± 0.1 ^{bc}
Red Lentil					
Non-micronized					
6%	5.3 ± 0.2 ^{a}	4.9 ± 0.4 ^{cd}	3.5 ± 0.6 ^{ab}	2.2 ± 0.0 ^{def}	4.6 ± 0.4 ^{c}
12%	4.9 ± 0.2 ^{abcd}	4.4 ± 0.1 ^{d}	3.9 ± 0.3 ^{a}	2.0 ± 0.3 ^{fg}	4.4 ± 0.2 ^{c}
Micronized					
6%	5.1 ± 0.3 ^{ab}	6.0 ± 0.2 ^{a}	1.6 ± 0.2 ^{ef}	2.7 ± 0.3 ^{a}	6.1 ± 0.1 ^{a}
12%	4.6 ± 0.1 ^{d}	4.8 ± 0.1 ^{cd}	2.8 ± 0.2 ^{bcd}	1.9 ± 0.3 ^{g}	4.8 ± 0.1 ^{bc}
Toasted Wheat Crumb					
6%	4.7 ± 0.1 ^{cd}	5.4 ± 0.5 ^{abc}	2.1 ± 0.6 ^{def}	2.5 ± 0.1 ^{abc}	5.5 ± 0.7 ^{ab}
Wheat Flour					
6%	4.9 ± 0.1 ^{abcd}	4.8 ± 0.5 ^{cd}	2.5 ± 0.6 ^{cde}	2.4 ± 0.0 ^{bcd}	5.0 ± 0.6 ^{bc}

[1]Means within the same column with the same letter are not significantly different ($p<0.05$).
[2] Highest possible score = 8 (Extremely desirable, intense flavour, high off-flavour, acceptable); Lowest possible score = 1 (Extremely undesirable, mild flavour, no off-flavour, unacceptable) except for saltiness.
[3] Highest possible score = 6 (Extremely salty); lowest possible score = 1 (Not detectable).

Other researchers have observed similar carry-over of legume flavour into meat applications. Prinyawiwatkul et al. (1997) reported undesirable flavour associated with the raw-beany, hay-like flavour of peanut flour (20%) to have transferred over to chicken nuggets, resulting in unacceptable flavour scores. However, the use of fermentation processes eliminated this carryover. Moreover, increasing the level of the binder ingredient can dilute the meat flavour as may have happened with the addition 12% binder.

Overall acceptability was rated highest for burgers with micronized green or red lentil at a level of an addition level of 6% (5.9 – 6.1). These burgers also exhibited the lowest off-flavour and flavour intensity, and the highest flavour desirability, and levels of juiciness comparable to those of reference burgers. Positive correlations between overall acceptability and flavour desirability (0.92, $p<0.0001$), and between overall acceptability and overall juiciness (0.36, $p<0.04$), and a negative correlation between overall acceptability and off-flavour (-0.79, $p<0.0001$), were found.

Generally, the effect of adding lentil flour to beef burger formulations was increased juiciness and tenderness and decreased hardness scores when compared to the control, although the addition of 12% of micronized lentil resulted in lower juiciness than in the control. Most notable is that micronization of lentil lowered or eliminated off-flavour development in burgers compared to those containing non-micronized lentil flour. The reduction in off-flavour development could be associated with the 100-fold decrease of lipoxygenase observed upon micronization of lentil seed (Table 4.2). The action of lipoxygenases is known to impart a bitter, cardboard taste through its oxidative mechanism (Sessa, 1979), and inactivation of this enzyme will prevent the development of these off-flavours.

4.2.7 Correlation coefficients for cooking properties, instrumental measurements, and sensory properties of low-fat beef burgers

Correlation coefficients can help to determine the strength of the relationships between data that was obtained from different types of tests (Meilgaard et al., 2007). There was a positive correlation ($p<0.001$) between cook yield and moisture retention ($r = 0.99$). Since the correlation between cook yield and fat retention was 0.32, this would indicate that moisture retention, rather than fat retention contributed more to the increase in cook yield as a result of binder addition to beef burgers. Moreover, increasing cook yields were positively correlated with overall tenderness ($r = 0.66$), and negatively correlated with shear force (-0.68), cohesiveness (instrumental, -0.76; and sensory, -0.75), and saltiness (-0.58) ($p<0.001$). This was expected as increased cook yield and the associated moisture retention, will offer higher tenderness and lower shear force, as well as serve to dilute the salts leading to lower saltiness. The lower cohesiveness with greater cook yield may be explained by a looser meat matrix as a result of greater moisture retention.

Correlations can also help in understanding the relationship between sensory and instrumental texture data (Meilgaard et al., 2007). The overall tenderness was negatively correlated ($p<0.001$) with the instrumental hardness as shown by texture profile analysis ($r = -0.88$) and shear force analysis ($r = -0.69$). Likewise, hardness from texture profile analysis was positively and negatively correlated ($p<0.001$) with the sensory scores for initial hardness ($r = 0.69$) and overall juiciness ($r = -0.81$), respectively. The significant strengths of these relationships indicate that instrumental texture analysis can produce results that are similar to tenderness and juiciness trends obtained from sensory techniques. Interestingly, texture as measured by compression testing (TPA) showed a stronger correlation ($r = 0.69$) to sensory hardness than by the shear force technique ($r = 0.53$); while the opposite was the case for overall tenderness where texture results by shear force analysis was more strongly correlated with overall tenderness ($r = -0.69$) than by compression testing ($p<0.001$).

Table 4.19: Correlation coefficients among cooking properties, instrumental texture measurements and semi-trained evaluations of low-fat beef burgers (n=33).

	Correlation Coefficients[1]												
	CY	**D**	**FR**	**MR**	**SF**	**TH**	**TCO**	**OJ**	**IH**	**OT**	**CO**	**FI**	**S**
Cook Properties													
Cook yield, CY	--												
Diameter, D	-0.76***	--											
Fat retention, FR	0.32	-0.23	--										
Moisture retention, MR	0.99***	-0.77***	0.28	--									
Texture Press Shear													
Shear force, SF	-0.68***	0.56***	-0.02	-0.72***	--								
Texture Profile Analysis													
Hardness, TH	0.25	-0.31	0.05	0.23	0.01	--							
Cohesiveness, TCO	-0.76***	0.34*	-0.24	-0.76***	0.64***	0.15	--						
Sensory Attributes													
Overall juiciness, OJ	-0.46**	0.37*	-0.36*	-0.43**	0.06	-0.81***	0.13	--					
Initial hardness, IH	-0.38*	0.14	-0.02	-0.39*	0.53***	0.69***	0.60***	-0.81***	--				
Overall tenderness, OT	0.66***	-0.39*	0.03	0.67***	-0.69***	-0.37*	-0.71***	0.30	-0.88***	--			
Cohesiveness, CO	-0.75***	0.50**	-0.28	0.76***	0.68***	0.16	0.71***	0.05	0.71***	0.81***	--		
Flavour intensity, FI	-0.21	0.04	-0.17	-0.17	-0.20	-0.33	0.17	0.56***	-0.36*	0.28	-0.06	--	
Saltiness, S	-0.58***	0.46**	-0.38*	-0.58***	0.37*	-0.28	0.33	0.55***	0.07	-0.15	0.38*	0.30	--

[1]Coefficients of three replications (n=33)

* $p<0.05$

** $p<0.01$

*** $p<0.001$

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4.2.8 Summary

In Part II, low-fat beef burgers were formulated with lentil- and wheat-based binders, and proximate, physical and cooking properties, instrumental texture, oxidative status, and sensory properties were analyzed. The burgers formulated contained <10% fat and decreasing levels of moisture as binder usage levels increased progressively from 0 to 12%. Upon cooking the burgers, increases in protein, fat, and ash were observed at the expense of lower moisture. As a result of cooking, there was a physical shrinkage in burger size accompanied by this moisture loss. Cooking yield increased to 86% as increasing binders levels up to 12% were incorporated into burgers. Moisture and fat retention analysis showed that this higher cooking yield was due to the water holding capacity of lentil, and not to its oil absorption capacity. This was apparent since moisture retention of burgers increased from 52 to 78% with increasing binder addition, whereas fat retention showed no differences among treatments. Despite the increases in WHC exhibited by burgers containing the micronized binders, micronized lentil flour addition to burgers had no effect on cooking yield. Levels of primary oxidative byproducts were highest in burgers (frozen) containing non-micronized lentil. When micronization was employed, TBARS decreased to the level of the control, or lower. There were no functionality differences observed with the use of green or red lentil flours as burger binders, except in the case of colour changes observed in the burgers with red lentil addition.

Despite the lower moisture content in burgers formulated with lentil flours, the perceived juiciness was improved at certain levels. Juiciness increased upon addition of 6% lentil binder, decreased when 12% was added, and further decreased when micronized lentil was used at 6 or 12%. Also, tenderness increased with 6 or 12% lentil addition.

When raw, fresh burgers were exposed to oxidative conditions (oxygen and light), the redness of raw burgers decreased over 7 days of refrigerated storage. In this experiment, the rate of decrease in a* was greatest for burgers containing non-micronized lentil, while those containing micronized lentil exhibited a lower rate of

decrease than the control. This indicates that components in the raw lentil offered pro-oxidant effects, while micronized lentil offered anti-oxidant effects.

Although off-flavour increased with lentil flour addition, it was reduced when lentil was micronized. Overall, green lentil had less impact on burger colour, and the 6% addition levels overall yielded more favourable sensory characteristics. Therefore, the use of green lentil flour and the micronizing conditions employed in this study (135 °C) were the most effective in creating a meat binder with similar impact as a reference binder. The resulting low-fat beef burgers had acceptable protein content, lower fat, and improved cooking properties, colour, juiciness, texture, and flavour qualities.

4.3 Study III: Consumer panel study of low-fat beef burgers

Consumer acceptability of burgers containing lentil flour was assessed by an un-trained panel. A consumer panel of 107 participants evaluated four burger treatments chosen based on the attributes such as level of juiciness, off-flavour, or colour as assessed in Study II. The large green lentil flour (dehulled) was chosen to represent a binder from this plant source since this treatment had minimal carryover of colour in the cooked burgers compared with the red lentil types. Moreover, this flour binder was investigated only at the 6% level, as previous results of 12% incorporation levels exhibited lower juiciness and texture qualities. In total, batches of four burger treatments were re-manufactured, consisting of a no-binder control, non-micronized and micronized green (large) lentil flour (6%), and toasted wheat crumb (6%) as an industry standard reference. The consumer panel completed a questionnaire providing demographic and consumer perception and behavioural information, which helped in the analysis of their sensory results.

4.3.1 Proximate composition of burgers

The proximate composition and pH of raw beef burgers produced for the consumer panel study are presented in Table 4.20. Values ranged from pH 5.6 to 5.8, 66.4 to 71.3% moisture, 17.0 to 17.8% protein, 7.9 to 8.8% fat, and 1.8 to 1.9% ash. These values are comparable to results determined for similarly formulated burgers that were used for the trained sensory panel in Part II. Although batches were produced on different dates, these similar proximate values were expected since the same meat cut and similar formulation and manufacturing protocols were used for both studies.

Table 4.20: pH[1] and proximate[1] values for raw, low-fat beef burgers used for the consumer panel.

Treatment	pH	Moisture (%)	Protein[2] (%)	Crude Fat (%)	Ash (%)
Control					
0%	5.6	71.3 ± 0.4	17.4 ± 0.2	8.8 ± 0.1	1.8 ± 0.1
Lentil Flour					
Non-micronized 6%	5.8	66.4 ± 0.1	17.8 ± 0.0	8.8 ± 0.0	1.9 ± 0.0
Micronized 6%	5.8	67.4 ± 0.3	17.8 ± 0.1	7.9 ± 0.4	1.8 ± 0.0
Toasted Wheat Crumb					
6%	5.7	67.6 ± 0.2	17.0 ± 0.1	8.2 ± 0.1	1.8 ± 0.0

[1]Values are means of duplicate determinations ± standard deviation
[2]Protein was calculated as total nitrogen x 6.25

4.3.2 Consumer demographics and purchasing behaviour

Consumer information regarding demographics and consumer beliefs and behaviour was obtained from Part II of the Questionnaire (Appendix 5). This information is important in understanding consumer familiarity with meat products prior to their participation and provides a basis for further consumer segmentation.

Tables 4.21 a, b, and c present consumer demographics and information about the purchase and consumption of meat products. On average, 91% of respondents consume burgers one time per month to two times per week, while 8% indicated that burgers are not typically consumed. The majority of consumers (63%) stated that they looked for lean or extra lean descriptors when purchasing ground beef and 81% of respondents indicated that they had purchased frozen burgers before. These results show that leaner beef meat is a common part of the diet, and that the majority of consumers has considered the convenience of beef burgers, and especially in their frozen form.

Table 4.21a: Consumer demographic data (n=107)

Question	Response	Percent (%)
How often do you consumer burgers?	3 - 5 times per week	1
	1 - 2 times per week	34
	1 - 2 times per month	57
	I don't eat burgers typically	8
	Total	100
What fat level of ground beef do you buy?	Regular Fat	16
	Lean	42
	Extra Lean	21
	I don't buy ground beef	7
	Other	14
	Total	100
Have you ever purchased frozen burgers?	Yes	81
	No	19
	Total	100

Table 4.21b: Consumer demographic data (n=107)

Question	Response	Percent (%)
Household Size	1	13
	2	35
	3	13
	4	21
	5	12
	6	2
	7	3
	9	1
	Total	100
Children Number	0	63
	1	17
	2	11
	3	6
	4	1
	5	1
	6	1
	Total	100
What is your role in grocery shopping for your household?	Primary shopper	52
	Share the shopping	34
	Someone else is the primary shopper	14
	Total	100
Household Income	Under 20,000	15
	20,000 - 39,000	15
	40,000 - 59,000	14
	60,000 - 79,000	17
	80,000 - 99,000	12
	Over 100,000	26
	Total	100

Table 4.21c: Consumer demographic data (n=107)

Question	Response	Percent (%)
Gender	Male	51
	Female	49
	Total	100
Age	18 - 29 Years	33
	30 - 39 Years	22
	40 - 49 Years	22
	50 - 59 Years	20
	60 - 69 Years	3
	Total	100
Education	Some High School	1
	High School Graduate	7
	Some University	21
	University College Graduate	31
	Graduate School	41
	Total	100
Ethnicity	European or North American	66
	Asian	17
	African	7
	Central or South American	7
	Other	4
	Total	100

Tables 4.21 b and c present consumer data relating to household and demographic information. Results show that households ranged in the number of occupants (1-9) with the greatest percentage (26%) of the households having gross annual incomes exceeding $100,000. The majority of the households (63%) did not have any children less than 18 years of age. About half (52%) of the respondents identified themselves as the primary shopper in their household, and 34% stated that they shared the shopping. Of the consumers surveyed, 31% held university degrees, while 41% had completed some level of graduate training. The majority (66%) of the consumer panel was of European/North American descent, 17% Asian, and 17% originating from other regions such as Africa, Central or South America. The age and sex distribution of panelists closely reflected that of the Canadian population (Statistics

Canada, 2008), except for the higher and lower numbers recruited in the 18 to 29 and 60 to 69 age categories, respectively, due to logistical circumstances of a university setting.

4.3.3 Consumer values

4.3.3.1 Product features

Understanding consumer perception of what factors are important when purchasing meat products is helpful for developing the ideal frozen low-fat beef burger. Table 4.22 displays various factors of importance and their consumer rating on a 3-point scale. This scale (1 = low importance, and 3 = high importance) has been reduced from an original 6-point scale for ease of review.

Table 4.22: Consumer statements on importance of product features when buying meat products (n = 107)

Purchasing	Importance[1]			
Factors	Low (%)	Medium (%)	High (%)	Total (%)
Price	14	53	33	100
Fat Content	16	35	49	100
Salt Content	24	39	38	100
Additive Exclusion	24	43	33	100
Flavour Variety	14	56	30	100
Meat Cut	28	44	28	100
Pre-Cooked Option	69	20	12	100

[1]Reduced to 3-point scale of importance from the original 6-point scale.

The majority of respondents indicated fat content to be the factor having the greatest importance, with price, salt, exclusion of additives, flavour variety, and meat cut having medium importance, and a pre-cooked option having the least importance (Table 4.22). Those factors having the greatest importance should be considered when developing products as they will have the greatest market impact. For example, lower salt in meat products has prompted positive attitudes, particularly in women (Guardia et al., 2006), and therefore, the advent of various studies investigating salt-reduced products. Likewise, studies investigating the potential use of non-meat adjuncts

(carrageenan, fibre, seed meal, starch-based) to replace fat in meat products are gaining prominence in food research (Desmond & Troy, 1998; Lin & Mei, 2000; Modi et al., 2009; Nowak et al., 2007; Pinero et al., 2008; Yilmaz & Daglioglu, 2003).

4.3.3.2 Beliefs and lifestyle

Understanding consumer beliefs and behaviour is important when developing a food product for a target market (Lawrence et al., 2003). Indicators of consumer beliefs and behavior in regards to health, nutrition, and food purchase are shown in Table 4.23. Three categories were used, with "1" signifying high disagreement, and "3" indicating high agreement. The majority of respondents considered themselves health conscious (42%), and exercised regularly (41%), read nutritional labels regularly (53%), and were willing to pay more for more nutritious foods (57%). Although 56% of respondents would choose lower fat food options when available, they would not necessarily do so at the expense of lower flavour quality, as 36% had strongly indicated this (41% neutral). Lastly, 74 and 65% of consumers believed that lentil and beef, respectively, are good sources of nutrition.

With current emphasis on reduced fat and salt levels, demand for nutritious food options while maintaining flavour quality, tendency towards a healthier lifestyle, and a positive perception of lentil and beef, a low-fat beef burger containing lentil could have potential marketability.

Table 4.23: Consumer belief and behavioural data (n=107)

Statement	Agreement Level			Total (%)
	Highly Disagree (%)	Neutral (%)	Highly Agree (%)	
I consider myself health conscious.	12	46	42	100
I exercise regularly.	20	39	41	100
I read food nutritional labels regularly.	20	27	53	100
I will pay more for nutritious foods.	11	31	57	100
Price is most important factor when buying meat products.	33	49	18	100
I choose lower fat versions of food when available.	13	29	58	100
I choose lower fat versions of food when available even at the expense of lower flavour quality.	36	42	22	100
I believe lentils are a good source of nutrition.	6	20	74	100
I believe that beef is a good source of nutrition.	3	32	65	100

4.3.4 Sensory scores for burgers

Average results for sensory attributes relating to aroma, juiciness, texture, and flavour intensity are shown in Table 4.24. Juiciness scores were lowest (3.8) for the no-binder control burger and the highest (4.7) for the burger containing non-micronized lentil. When the binder had been micronized, burger juiciness decreased significantly (4.4) which was comparable to the effect of toasted wheat crumb on juiciness (4.4). The same trend for the effect of micronized lentil flour on burger juiciness was also observed in the study using trained panelists (Part II). As described previously, lower juiciness in burgers with heat-treated lentil flour may be due to the water bound by the micronized lentil flour being unavailable. The presence of free water in the cooked burger contributes to meat juiciness (Aberle et al., 2001), and is apparent in the release of liquid upon initial bite. There were no significant score differences ($p<0.05$) for aroma (3.2 –

3.3) and flavour intensity (3.9 – 4.2) among the four burger treatments according to the consumer panelists.

Table 4.24: Mean consumer sensory scores[1,2] for four burger treatments (n=107).

Treatment	Sensory Attribute[3]			
	Aroma	**Juiciness**	**Texture**	**Flavour Intensity**
Control				
0%	3.3 ± 1.3 [a]	3.8 ± 1.2 [c]	3.7 ± 1.2 [c]	3.9 ± 1.0 [a]
Lentil Flour				
Non-micronized 6%	3.3 ± 1.4 [a]	4.7 ± 0.9 [a]	5.0 ± 0.9 [a]	4.2 ± 1.1 [a]
Micronized 6%	3.3 ± 1.3 [a]	4.4 ± 1.0 [b]	4.7 ± 1.0 [b]	4.1 ± 0.9 [a]
Toasted Wheat Crumb				
6%	3.2 ± 1.2 [a]	4.4 ± 1.0 [b]	4.8 ± 0.9 [ab]	4.1 ± 1.0 [a]

[1]Values are means of 107 scores ± standard deviation.
[2]Means with different superscript within each column are significantly different ($p<0.05$).
[3]Highest possible score = 6 (Very strong, juicy, tender, intense);
Lowest possible score = 1 (Very mild, dry, tough, bland)

The consumer panel found the texture of the burger with no-binder (control) to be the toughest (3.7), and the burger with non-micronized lentil flour was most tender (5.0) ($p<0.05$). Interestingly, burgers with micronized lentil were significantly more tender than when not micronized ($p<0.05$), which was different from the results found by the trained panel where micronization had no effect on burger tenderness. This difference could be due to the lack of training in consumer panel analysis. A positive relationship ($r = 0.73$, $p<0.0001$) was found between tenderness and juiciness as was observed in a consumer study by Kukowski et al. (2004), who evaluated consumer acceptability and sensory scores of different beef cuts. Juiciness and tenderness was more positively correlated ($r = 0.60$, $p<0.001$, not shown) in the current consumer panel study than in the trained panel study ($r = 0.30$, $p<0.1$, Table 4.19).

4.3.5 Acceptability scores for burgers

Sensory scores for the acceptability of the four burger treatments are displayed in Table 4.25. The acceptability scores for juiciness and texture were lowest in the no-binder control ($p<0.05$). Flavour acceptability was higher in burgers with micronized lentil (4.7) compared with those with non-micronized lentil or the no-binder control (4.2), and was similar to those with TWC (4.5). There were no significance differences in aroma acceptability ($p<0.05$).

Table 4.25: Mean acceptability scores for four burger treatments (n = 107).

Treatment	Acceptability[3]			
	Aroma	**Juiciness**	**Texture**	**Flavour**
Control				
0%	4.2 ± 1.0 [a]	4.1 ± 1.2 [b]	4.0 ± 1.1 [b]	4.2 ± 1.1 [b]
Lentil Flour				
Non-micronized 6%	4.4 ± 1.0 [a]	4.6 ± 1.1 [a]	4.8 ± 0.9 [a]	4.2 ± 1.2 [b]
Micronized 6%	4.4 ± 1.1 [a]	4.7 ± 1.0 [a]	4.9 ± 0.8 [a]	4.7 ± 0.9 [a]
Toasted Wheat Crumb				
6%	4.3 ± 1.0 [a]	4.8 ± 0.9 [a]	4.8 ± 0.9 [a]	4.5 ± 1.1 [ab]

[1]Values are means of 107 scores ± standard deviation.
[2]Means with different superscript within each column are significantly different ($p<0.05$).
[3]Highest possible score = 6 (Extremely like); Lowest possible score = 1 (Extremely dislike)

Although the burger with non-micronized lentil yielded high juiciness and tenderness scores (Table 4.24), the flavour acceptability was the lowest (Table 4.25). The acceptability of juiciness and texture of burgers containing non-micronized lentil were not significantly different from those with micronized lentil, yet the acceptability of these two burger treatments was significantly different in the case of flavour acceptability. The overall acceptability was highest in burgers with micronized lentil or with TWC (Table 4.25). This indicates that flavour characteristics played a major role in the assessment of overall acceptability of the burgers. As a result, the frequency of a "yes" response to "willingness to purchase product" was more prevalent in burgers with

micronized lentil or toasted wheat crumb, compared with burgers with either non-micronized lentil or no binder (Table 4.26). Off-flavours were observed in various legume-meat applications including cowpea and peanut flours in chicken nuggets (Prinyawiwatkul et al., 1997), and chickpea meal in meat loaves (Shaner & Baldwin, 1979), and a decrease in hedonic flavour scores was observed when up to 4% soy, and bengal, green, and black gram flours were added to buffalo burgers. Conversely, no flavour differences were detected in meat loaves extended with up to 30% of textured soy (Williams & Zabik, 1975), or in beef burgers formulated with 0.5% pea fibre concentrate (Besbes et al., 2008) compared to a control.

Table 4.26: Overall acceptability and willingness to purchase scores[1,2,3] for four burger treatments (n = 107).

Treatment	Overall Acceptability	Willingness to Purchase[4]
Control		
0%	4.1 ± 1.0 [b]	1.5 ± 0.5 [a]
Lentil Flour		
Non-micronized 6%	4.4 ± 1.2 [b]	1.4 ± 0.5 [a]
Micronized 6%	4.7 ± 0.9 [a]	1.2 ± 0.4 [b]
Toasted Wheat Crumb		
6%	4.7 ± 1.0 [a]	1.2 ± 0.4 [b]

[1]Values are means of 107 scores ± standard deviation.
[2]Means with different superscript within each column are significantly different ($p<0.05$).
[3] Highest possible score = 6 (Extremely like); Lowest possible score = 1 (Extremely dislike)
[4] 1 = yes; 2 = no

4.3.5.1 Consumer segmentation

In this study, classification of consumers according to their demographics yielded significantly different average responses in burger acceptability compared to those obtained when averages were calculated from all consumers. The mean acceptability scores categorized by gender for burgers treatments are presented in Table 4.27. Means with different superscripts within each row are significantly different ($p<0.05$). When gender is taken into account, males found that burgers with micronized lentil were more acceptable (4.7) than if non-micronized lentil was used as a binder (4.3). On the other hand, females found no difference between these two burger treatments ($p<0.05$), and that the no-binder control was significantly less acceptable (3.8) than all other treatments. Males generally found the no-binder control (4.3) and toasted wheat crumb reference burger (4.8) to be more highly acceptable than did females (3.8, 4.5, respectively).

Table 4.27: Mean acceptability scores[1,2] for burgers containing various binders according to gender (n=107).

Gender	Percent of panelists	Burger Treatment			
		Control	GLO	GLM	TWC
Male	51	4.3 ± 1.0 [b]	4.3 ± 1.2 [b]	4.7 ± 0.9 [a]	4.8 ± 0.7 [a]
Female	49	3.8 ± 1.0 [b]	4.4 ± 1.2 [a]	4.7 ± 0.9 [a]	4.5 ± 1.2 [a]

[1]Means with different superscript within each row are significantly different ($p<0.05$).
[2] Highest possible score = 6 (Extremely like);
Lowest possible score = 1 (Extremely dislike)
(GLO = non-micronized green lentil; GLM = micronized green lentil; TWC = toasted wheat crumb)

The mean acceptability scores for burgers treatments when categorized by age groups are presented in Table 4.28. Means with different superscripts within each row are significantly different ($p<0.05$). The youngest consumer group (ages 18 to 29) found burgers with non-micronized lentil to be significantly lower in acceptability than if micronized lentil was used. In comparison, all other age groups found no significant difference between these two treatments. Taste sensitivity in this age segment may be greater or familiarity with foreign flavours is lower. Moreover, the 18 to 29 and 50 to 59 age groups found the no-binder control burger to have acceptability comparable to all other treatments, while all other age groups found the no-binder control to have significantly lower acceptability than other treatments.

Table 4.28: Mean acceptability scores[1,2] for burgers containing various binders according to age group (n=107).

Age (Years)	Percent of panelists	Burger Treatment			
		Control	GL	GLM	TWC
18 - 29	33	4.1 ± 1.0 [ab]	4.0 ± 1.1 [b]	4.6 ± 1.1 [a]	4.6 ± 1.2 [a]
30 - 39	22	4.1 ± 1.0 [b]	4.8 ± 1.2 [a]	4.8 ± 1.1 [a]	4.6 ± 1.1 [ab]
40 - 49	22	4.3 ± 1.0 [b]	4.7 ± 1.0 [ab]	4.9 ± 0.4 [a]	5.0 ± 0.7 [a]
50 - 59	20	4.0 ± 1.2 [a]	4.1 ± 1.3 [a]	4.5 ± 0.8 [a]	4.6 ± 0.9 [a]
60 - 69	3	3.3 ± 0.6 [b]	4.7 ± 0.6 [a]	4.7 ± 0.6 [a]	4.7 ± 0.6 [a]

[1]Means with different superscript within each row are significantly different ($p<0.05$).

[2] Highest possible score = 6 (Extremely like); Lowest possible score = 1 (Extremely dislike)

GL = Large green lentil, non-micronized; GLM = Large green lentil, micronized; TWC = Toasted wheat crumb

It was shown that categorizing consumers according to specific characteristics could identify significant differences in acceptability of burgers within these subgroups. Using mean values for the entire sample would have overlooked these differences. These varying hedonic acceptance scores among different consumer segments can

reveal consumer food preferences and could, therefore, serve as a basis for customized marketing strategies.

4.3.6 Summary

A consumer panel of 107 participants was used to provide consumer demographic, perceptual, and behavioural information, as well as to evaluate four burger selections containing no binder, non-micronized and micronized green lentil flour, and toasted wheat crumb, respectively. Most consumers were familiar with burgers and stated that nutrition and flavour were the important attributes to this food and would adjust purchasing behaviour accordingly. Consumers found the juiciness and texture of burgers with micronized lentil to be comparable to burgers containing toasted wheat crumb, but less juicy and tender than those containing non-micronized lentil. Although burgers with non-micronized lentil were more juicy and tender, flavour acceptability of the burgers with micronized lentil was higher than the non-micronized or the control, and comparable to those containing toasted wheat crumb. Consumer segmentation by age and gender showed that hedonic acceptability scores varied by different categories for the four burger treatments. Females found burgers with micronized and non-micronized lentil to have similar acceptability, whereas males or consumers falling within the 18 to 29 age group found burgers with non-micronized lentil to be less acceptable than those with micronized lentil.

5. OVERALL SUMMARY AND CONCLUSIONS

The overall objective of this study was to assess the potential for using flour from micronized lentil as a binder in a low-fat beef burger application. Concerns with using binders as a fat reduction strategy in meat products include effects on composition, juiciness, texture, colour, oxidative stability, and flavour. It was shown in this project that the micronizing conditions (tempering seed to 15% moisture and heating to a surface temperature of 135 °C) influenced the compositional, physical, functional, and thermal properties of dehulled lentil. These effects were measured by analyzing non-micronized and micronized lentils for their proximate composition, degree of gelatinization, lipoxygenase activity, particle size distribution, colour, nitrogen solubility, water holding and oil absorption capacity, and thermal behaviour using viscometry and calorimetry. Subsequently, flours from green and red lentil (large type) were incorporated into low-fat beef burgers at levels of 6 and 12%. Proximate composition, cook properties, instrumental texture, colour, oxidative quality, and sensory analysis were conducted. Comparisons were made to burgers containing wheat flour, or toasted wheat crumb which is used commercially.

In Part I, it was shown that tempering and micronizing had an impact on the physiochemical properties of dehulled lentil. With protein and starch comprising a large proportion of the lentil seed, changes in these components were monitored. The intensity of the micronization treatment on lentils was sufficient for pre-gelatinizing some of the starch while not fully denaturing the proteins. Therefore, the degree of protein denaturation and the proportion of starch gelatinized were shown to be adequate in terms of yielding characteristics that constitute a suitable meat binder. This allowed for increases in WHC, which is a favourable property for ingredients used in low-fat meat products in which the retained moisture can offer mouth-feel characteristics similar to those of fat. Although there was no improvement in OAC due to micronization of

lentil, this was not important in the case of low-fat beef burgers because the differences in fat loss upon cooking among burgers with and without binders were not significant. Furthermore, micronization was effective in reducing LOX enzyme activity in lentil which is of practical significance in terms of shelf life. Micronization resulted in lentil flours with enhanced properties, which made them more suitable as a meat binder.

The functional characteristics of flours from micronized and non-micronized lentil were demonstrated as 0 to 12% of binder was added to low-fat beef burger formulations. In general, lentil flour increased cooking yield (up to 12%) and overall juiciness sensory scores (up to 6% level), trends similar to those exhibited by burgers with commercial toasted wheat crumb (6%). However, the use of micronized lentil flour was critical in enhancing the properties of low-fat burgers containing lentil flour. Although greater crust formation was evident in burgers with 6 or 12% micronized lentil, interior texture was less "mushy" than if the flour was from non-micronized lentil. Moreover, the use of micronized lentil flour in burgers exhibited suppression of oxidation when the raw burgers were stored under refrigeration over 7 days, which corresponded to significantly lower TBARS (when raw burgers were stored frozen for approximately 10 weeks) compared with the effects of non-micronized lentil. These results have shelf life implications especially in the development of raw, frozen burgers. When burgers were cooked, there was carry-over of redness from the red lentil flour but this colour effect was reduced if the flour was from micronized red lentil. Most distinctive was the significant decrease in off-flavour in burgers with micronized lentil flour compared to those with non-micronized lentil flour. These flavour differences also were detected by a consumer panel (n=107), which contributed to a positive increase in the overall acceptability of burgers with micronized lentil (6%), which was comparable to the burgers with toasted wheat crumb.

Collectively, these results demonstrate that micronization of dehulled lentil to a surface temperature of 135 °C altered the functional properties of the flour, and was effective in enhancing juiciness, texture, flavour, oxidative stability, and colour of low-fat beef burgers. Therefore based on these results, micronized lentil flour has potential as a functional binder in the meat burger industry at levels complying with the protein

source requirements of the CFIA. Future studies could be directed to the area of investigating the effects of different micronization conditions on lentil flour functionality and their subsequent application in a wider range of food systems.

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Appendix 1

Participant Name and ID: _____
Date: _____
Booth#: _____

Sample Code:

Sensory Evaluation of Cooked Beef Burgers

Evaluate samples in the order that the sample codes on the score cards are arranged. Please take a bite of cracker and a drink of water **before** and **between** sampling. Please **CIRCLE** a descriptor along the 8-point scale for each characteristic.
Feel free to list comments at the bottom of this page. Use the back of this page if necessary.

	8	7	6	5	4	3	2	1
Initial Juiciness	Extremely juicy	Very juicy	Moderately juicy	Slightly juicy	Slightly dry	Moderately dry	Very dry	Extremely dry
Initial Hardness	Extremely hard	Very hard	Moderately hard	Slightly hard	Slightly soft	Moderately soft	Very soft	Extremely soft
Cohesiveness	Extremely cohesive	Very cohesive	Moderately cohesive	Slightly cohesive	Slightly loose	Moderately loose	Very loose	Extremely loose
Saltiness	Extremely salty	Very salty	Moderately salty	Slightly salty	Very slightly salty	Not detectable		
Overall Tenderness	Extremely tender	Very tender	Moderately tender	Slightly tender	Slightly tough	Moderately tough	Very tough	Extremely tough
Overall Juiciness	Extremely juicy	Very juicy	Moderately juicy	Slightly juicy	Slightly dry	Moderately dry	Very dry	Extremely dry
Flavour Desirability	Extremely desirable	Very desirable	Moderately desirable	Slightly desirable	Slightly undesirable	Moderately undesirable	Very undesirable	Extremely undesirable
Overall Flavour Intensity	Extremely intense	Very intense	Moderately intense	Slightly intense	Slightly mild	Moderately mild	Very mild	Extremely mild
Presence of Off-Flavour *(Describe below)*	Extremely high	Very high	Moderately high	Slightly high	Slightly low	Moderately low	Very low	None
Overall Acceptability	Extremely acceptable	Very acceptable	Moderately acceptable	Slightly acceptable	Slightly unacceptable	Moderately unacceptable	Very unacceptable	Extremely unacceptable

Comments:

Appendix 2

Trained Sensory Panel

Definition of Sensory Attributes

First Bite

Take a bite of a warm burger sample with your front teeth and evaluate the initial JUICINESS and HARDNESS

Initial Juiciness	Amount of juice released as teeth apply pressure
Initial Hardness	Amount of force required to bite through sample

During Mastication

Subsequently, move the mass to the centre of the mouth and using your tongue as a feeler, evaluate for COHESIVENESS. Rate the SALT intensity.

Cohesiveness	Degree to which particles stick together after 7 to 8 chews
Saltiness	Degree of salt intensity

End of Mastication

Before swallowing, evaluate for overall TENDERNESS, JUICINESS, and FLAVOUR.

Overall Tenderness	Amount of time and effort required to completely chew sample before swallowing
Overall Juiciness	Amount of moisture in your mouth at the end of chewing
Flavour Desirability	Degree of liking of flavour

After Swallowing

Overall Acceptability	Degree of acceptability of product

Appendix 3

Sample Code:

Participant # : _____
Order #: _____

PART I: Consumer Sensory Evaluation Beef Burgers

Please evaluate the samples in the order that the sample codes (top right) are presented to you. Please take a bite of cracker and a drink of water **before** and **between** sampling.

1) Check **ONE** descriptor along the 6-point scale for each sensory characteristic.

	6	5	4	3	2	1
Aroma	Very Strong ☐	Moderately Strong ☐	Slightly Strong ☐	Slightly Mild ☐	Moderately Mild ☐	Very Mild ☐
Acceptability of Aroma	Like Extremely ☐	Like Moderately ☐	Like Slightly ☐	Dislike Slightly ☐	Dislike Moderately ☐	Dislike Extremely ☐
Juiciness	Very Juicy ☐	Moderately Juicy ☐	Slightly Juicy ☐	Slightly Dry ☐	Moderately Dry ☐	Very Dry ☐
Acceptability of Juiciness	Like Extremely ☐	Like Moderately ☐	Like Slightly ☐	Dislike Slightly ☐	Dislike Moderately ☐	Dislike Extremely ☐
Texture	Very Tender ☐	Moderately Tender ☐	Slightly Tender ☐	Slightly Tough ☐	Moderately Tough ☐	Very Tough ☐
Acceptability of Texture	Like Extremely ☐	Like Moderately ☐	Like Slightly ☐	Dislike Slightly ☐	Dislike Moderately ☐	Dislike Extremely ☐
Flavour Intensity	Very Intense ☐	Moderately Intense ☐	Slightly Intense ☐	Slightly Bland ☐	Moderately Bland ☐	Very Bland ☐

-- Continue to Next Page --

	6	5	4	3	2	1
Acceptability of Flavour	Like Extremely ☐	Like Moderately ☐	Like Slightly ☐	Dislike Slightly ☐	Dislike Moderately ☐	Dislike Extremely ☐
Overall Acceptability	Like Extremely ☐	Like Moderately ☐	Like Slightly ☐	Dislike Slightly ☐	Dislike Moderately ☐	Dislike Extremely ☐

1) Please indicate what you liked or disliked about this product.

LIKED	DISLIKED

2) Would you buy this product? *(Check one)*

☐ Yes ☐ No

- **Please review that you have answered all the questions above.**
- **You may now proceed to the next sample.**
- **If this is your last sample, push your tray forward and close the hatch. You may now proceed to PART II: Consumer Survey.**

Appendix 4

Consumer Sensory Panel

Definition of Sensory Terminologies

Before First Bite

Before the first bite into the sample, smell the burger sample and assess the aroma intensity and aroma desirability.

Aroma	Odour, smell

First Bite

Juiciness	Amount of juice released as teeth apply pressure
Texture	Amount of time and effort required to completely chew sample before swallowing
Flavour Intensity	Degree of flavour released from burger while chewing

After Swallowing

Overall Acceptability	Degree of acceptability of product

Appendix 5

Participant # _____

PART II: Beef Burger Consumer Survey

Please answer the questions below. The information will be treated with strict confidence and you will not be asked to identify yourself on the survey.

On average, how often do you consume burgers?

- ☐ 3 - 5 times per week
- ☐ 1 - 2 times per week
- ☐ 1 - 2 times per month
- ☐ I don't eat burgers typically

In which setting do you consume burgers? *(Check all that apply)*

- ☐ 'Sit-down' restaurant service
- ☐ 'Take-out' from a restaurant
- ☐ At home - made from ground meat
- ☐ At home - cooked from store-bought frozen burgers
- ☐ Other: _____

When purchasing ground beef, which fat level would you typically purchase? *(Check all that apply)*

- ☐ Regular fat
- ☐ Lean
- ☐ Extra lean
- ☐ I don't buy ground beef

Have you ever purchased frozen burgers? ☐ Yes ☐ No

If you answered YES to the previous question, please indicate how important the following features are to you when shopping for frozen burgers.

	Not at all important ⟷ **Extremely Important**					
	1	2	3	4	5	6
Price	☐	☐	☐	☐	☐	☐
Fat content	☐	☐	☐	☐	☐	☐
Salt content	☐	☐	☐	☐	☐	☐
Exclusion of additives	☐	☐	☐	☐	☐	☐
Variety of flavours	☐	☐	☐	☐	☐	☐
Made from a specific meat cut, e.g. sirloin, prime rib	☐	☐	☐	☐	☐	☐
Pre-cooked option	☐	☐	☐	☐	☐	☐

Below is a list of statements relating to food purchasing habits and lifestyle. For each, please indicate how much you agree or disagree on the scale provided.

	Completely Disagree ⟷ Completely Agree					
	1	2	3	4	5	6
I consider myself very health conscious.	☐	☐	☐	☐	☐	☐
I exercise on a regular basis.	☐	☐	☐	☐	☐	☐
I regularly read nutritional labels on the food I purchase.	☐	☐	☐	☐	☐	☐
I will pay more for a food product if it is more nutritious than a cheaper alternative.	☐	☐	☐	☐	☐	☐
Price is the most important factor I consider when I buy meat products.	☐	☐	☐	☐	☐	☐
I prefer to buy the lower-fat version of a food product if it is available.	☐	☐	☐	☐	☐	☐
I will opt for the lower-fat version of a food product, even at the expense of lower flavour quality.	☐	☐	☐	☐	☐	☐
I believe that lentils are a good source of nutrition.	☐	☐	☐	☐	☐	☐
I believe that beef is a good source of nutrition.	☐	☐	☐	☐	☐	☐

The following questions are intended to understand the general demographics of participants. The information will be kept confidential and will only be used to understand broad trends, and not on an individual level.

How many people live in your home including yourself?
Enter number: _____

How many children (< 18 years old) live in your home?
Enter number: _____

Which of the following categories best describes your role in grocery shopping for your household?

- ☐ Primary shopper
- ☐ Share the shopping
- ☐ Someone else is the primary shopper

Which one of the following best describes your annual household income level before taxes?

- ☐ Under $20,000
- ☐ $20,000 - 39,000
- ☐ $40,000 - 59,000
- ☐ $60,000 - 79,000
- ☐ $80,000 - 99,000
- ☐ Over $100,000

Gender

- ☐ Male
- ☐ Female

Age Category

- ☐ 18 - 29 years
- ☐ 30 - 39 years
- ☐ 40 - 49 years
- ☐ 50 - 59 years
- ☐ 60 - 69 years
- ☐ Over 70 years

Education *(Highest level completed)*

- ☐ Some Grade School
- ☐ Some High School
- ☐ High School Graduate
- ☐ Some University
- ☐ University/College Graduate
- ☐ Graduate School

Ethnic Background *(Check all that apply)*

- ☐ European
- ☐ North American
- ☐ First Nation
- ☐ Asian
- ☐ African
- ☐ Central / South American
- ☐ Other

This is the END of the survey. Thank you for participating in this study.

Please hand in your completed Score Sheet and Consumer Survey as you exit. Feel free to help yourself to some treats.